



NANOPARTICLES IN DRUG DELIVERY

Lovisa Ringstad, PhD

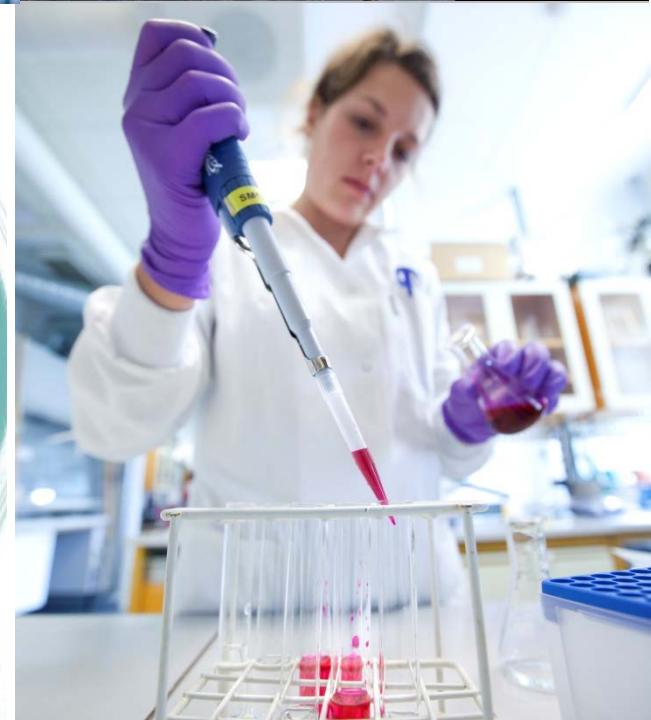
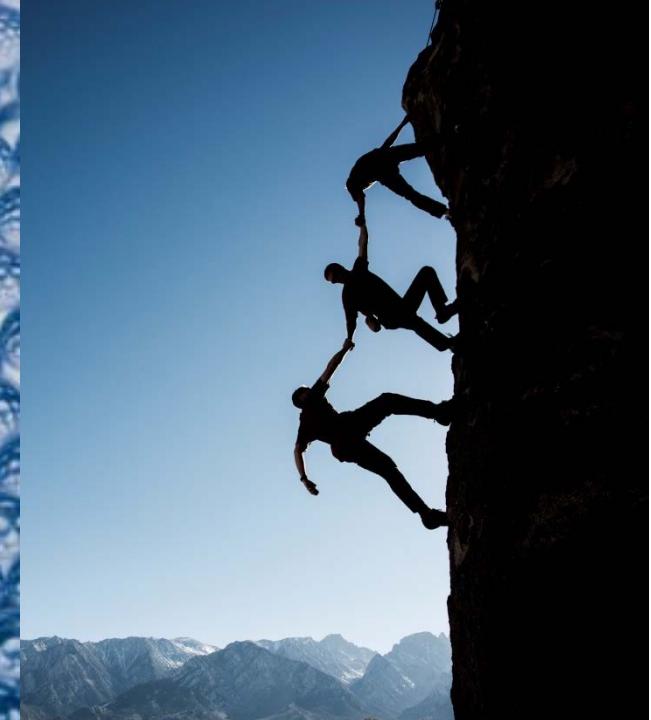
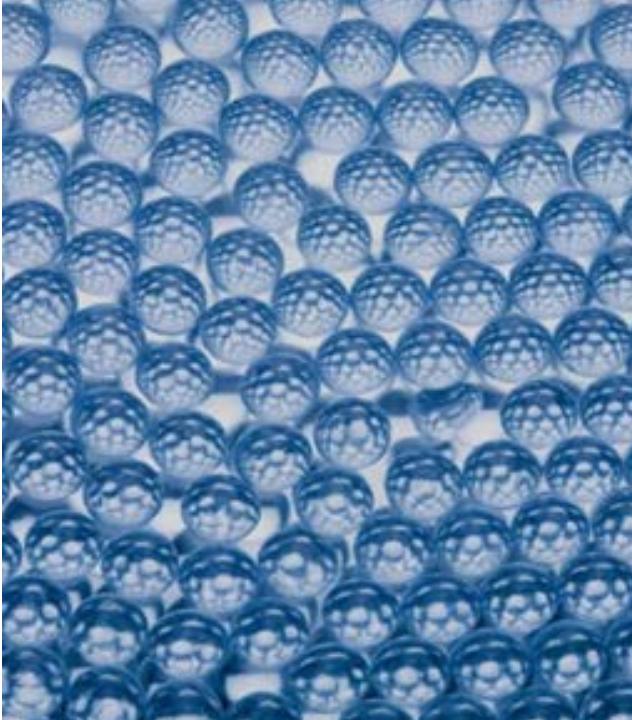
Deputy Head of Section, Formulation Development

BB2170 Course in Drug Development

Sept 28, 2018

Research Institutes of Sweden

Bioscience and Materials
Surface, Process and Formulation



Outline

- RISE Research Institutes of Sweden
- Nano in drug delivery
 - Different strategies
 - Some examples
- Infectious diseases and antimicrobial resistance
- FORMAMP - a nanoformulation project

RISE in brief

- Present across the whole of Sweden. And beyond.
- 2,300 employees, 30 % with a PhD.
- Turnover approx. SEK 2.7 billion (2017).
- A large proportion of customers are SME clients, accounting for approx. 30 % industry turnover.
- Runs 100s of test and demonstration facilities, open for industry, SMEs, universities and institutes (RISE is owner and partner in 60 % of all Sweden's T&D facilities).



Our vision

An internationally
leading partner for
innovation



With our broad range of **competencies** and **unique expertise**, we create added value



Cement and concrete



Certification



Circular economy



Design



Electronics



Energy and fuels



Packaging



Glass



Health and Care



ICT and telecoms



Agriculture and food



Chemistry, materials and surfaces



Life Science



Maritime



Mechanical engineering



Mechanics



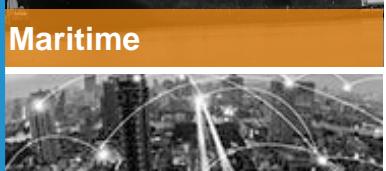
Metrology and measurement technology



Paper and Pulp



Process development



Built environment



Safety



Mobility



Wood



Water



Bioeconomy



Fire and safety

Our combined offer

▪ **Applied Research and Development**

- Research and Innovation projects
- Expert consultation
- Service design and design processes
- Innovation support for SMEs

▪ **Industrialisation and Verification**

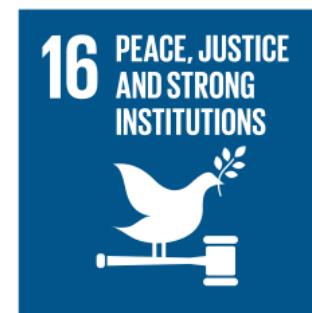
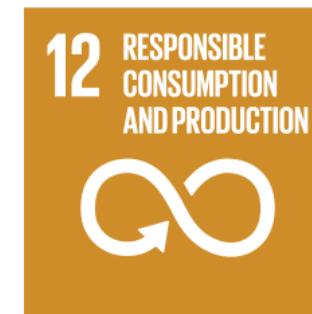
- Testbeds and demonstration facilities
- Technical assessments and verification
- Prototypes and pilot line production

▪ **Quality Assurance**

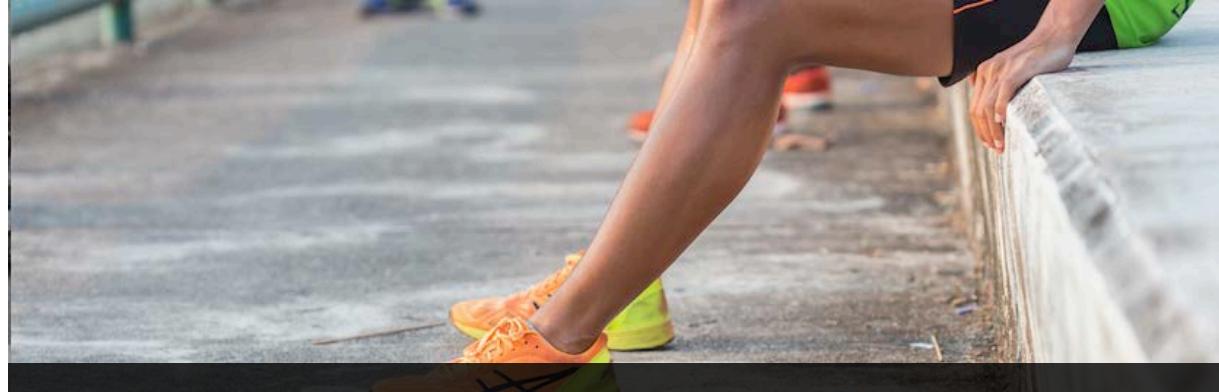
- Certification



Sustainability – a central pillar of our business strategy



Health and Life Science – focus areas



Prevention



E-Health



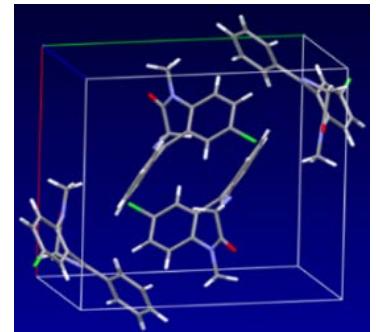
New therapies



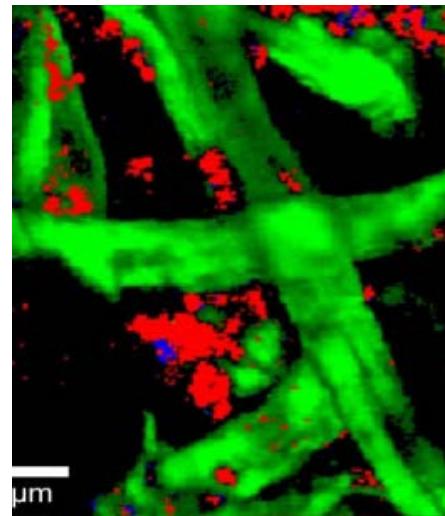
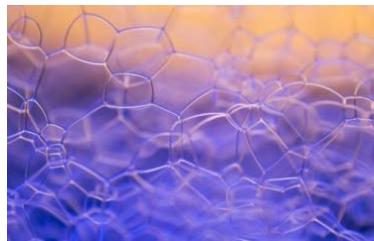
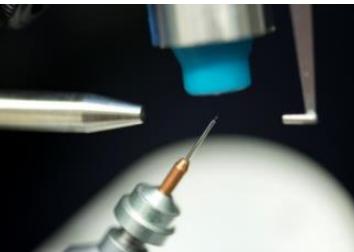
Infection Control



RISE Surface, Process and Formulation



Our Focus:
Research, Development and Innovation within the areas of
Surface Design, Chemical Processes, Material Development,
Formulation Sciences and Pharmaceutical Development

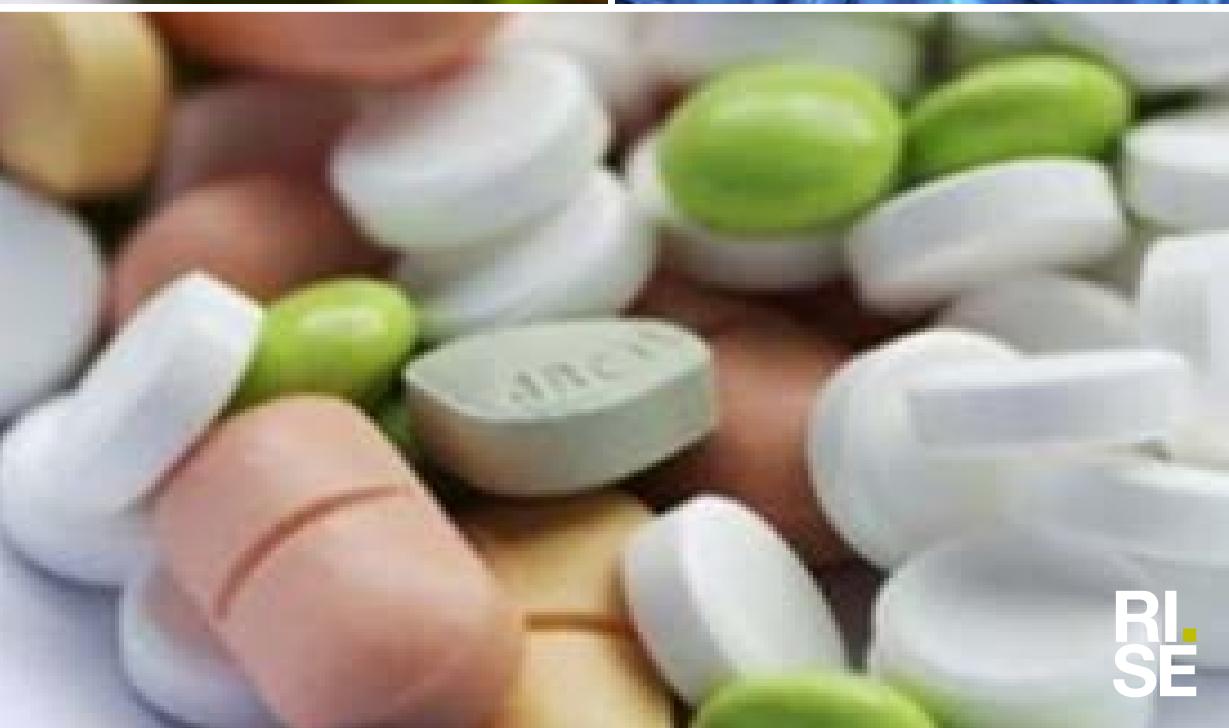


Our industrial areas

We operate in a wide range of industries.

The 5 industry sectors with most projects in our portfolio are:

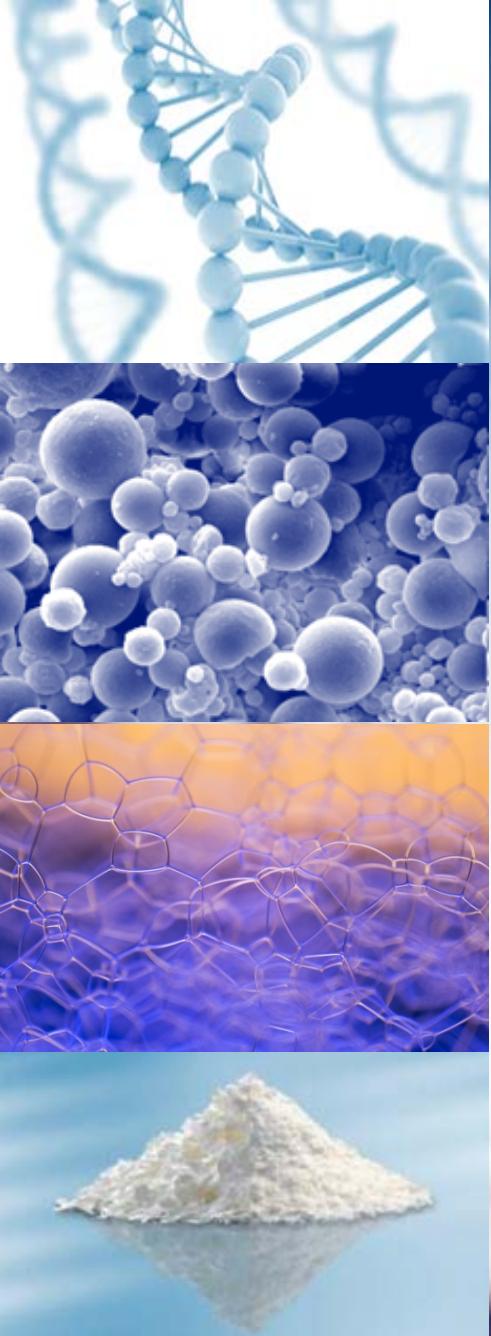
- Pharmaceuticals and Medtech
- Chemical industry
- Energy (including Bioeconomy)
- Home and personal care
- Food



Our Competence Areas – *Propel Your Innovation*

- Pharmaceutical Development
- Process Development & Scale Up
- Synthetic & Material Chemistry
- Chemical Engineering
- Analytical Sciences
- Formulation Sciences
- Surface Design





- Need-driven research and innovation in the formulation area
- Mission: Improve performance of formulations resulting in sustainable products, reduced side-effects and improved health
- **Focus areas:** Formulation of biologics, Controlled delivery and release, Powder technology and Emulsions and disperse systems
- Collaborative projects, networking, courses
- www.rise-perform.com

Contact: Isabel Mira, isabel.mira@ri.se

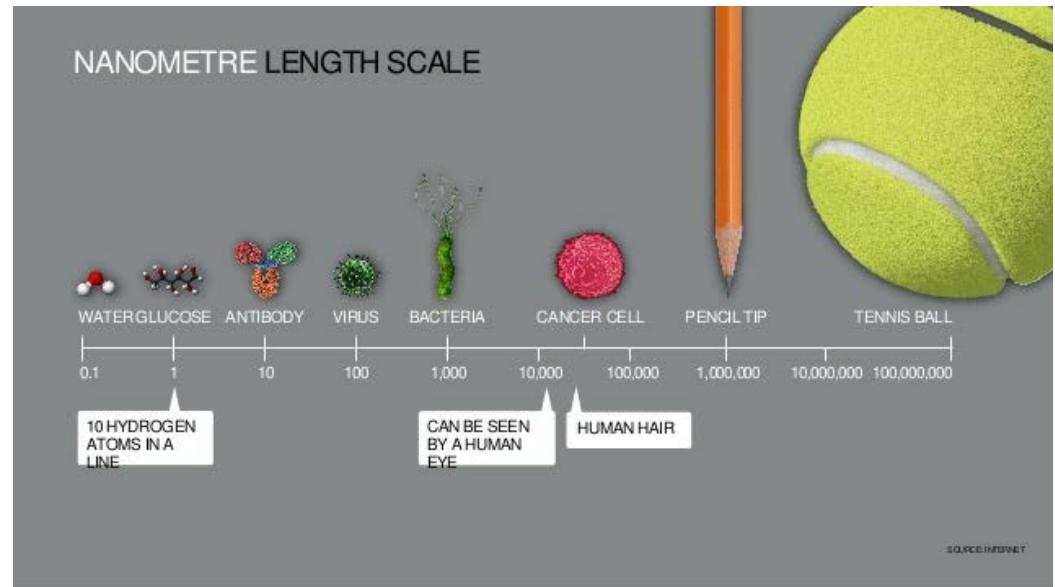
Nanomedicine



https://www.youtube.com/watch?feature=player_embedded&v=2VcNpl8-PRI

What is Nano?

- $0.000000001 = 10^{-9}$
- Nano means dwarf in Greek
- Nanoscale devices are 100–10,000 times smaller than human cells
- A human hair is about 80,000 nanometers in diameter
- A DNA molecule is between 2 and 12 nanometers wide
- 1 nm is about the length that a fingernail grows in 1 s



'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm.

<http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32011H0696>

Nanotechnology is the understanding and control of matter at dimensions between approx. 1-100 nm, where unique phenomena enable novel applications not feasible when working with bulk materials or even with single atoms or molecules

NHI NNI definition
(<https://www.nano.gov/nanotech-101/nanotechnology-facts>)

Nanopharmaceuticals is pharmaceuticals engineered on the nanoscale, i.e. pharmaceuticals where the nanomaterial plays the pivotal therapeutic role or adds additional functionality to the previous compound.

Rivera et al Pharmacol Res 2010;62(2):115-125

Nanomedicine: use of nanotechnology to achieve innovation for healthcare applications

Why nanoparticles in medicine?

- Extremely small size
- High surface area
- Surface modification - Functionalization
- High bioavailability
- Biological barriers
- Passive and targeted delivery
- Controlled release
- Administration via different routes
- Hydrophilic and hydrophobic drugs



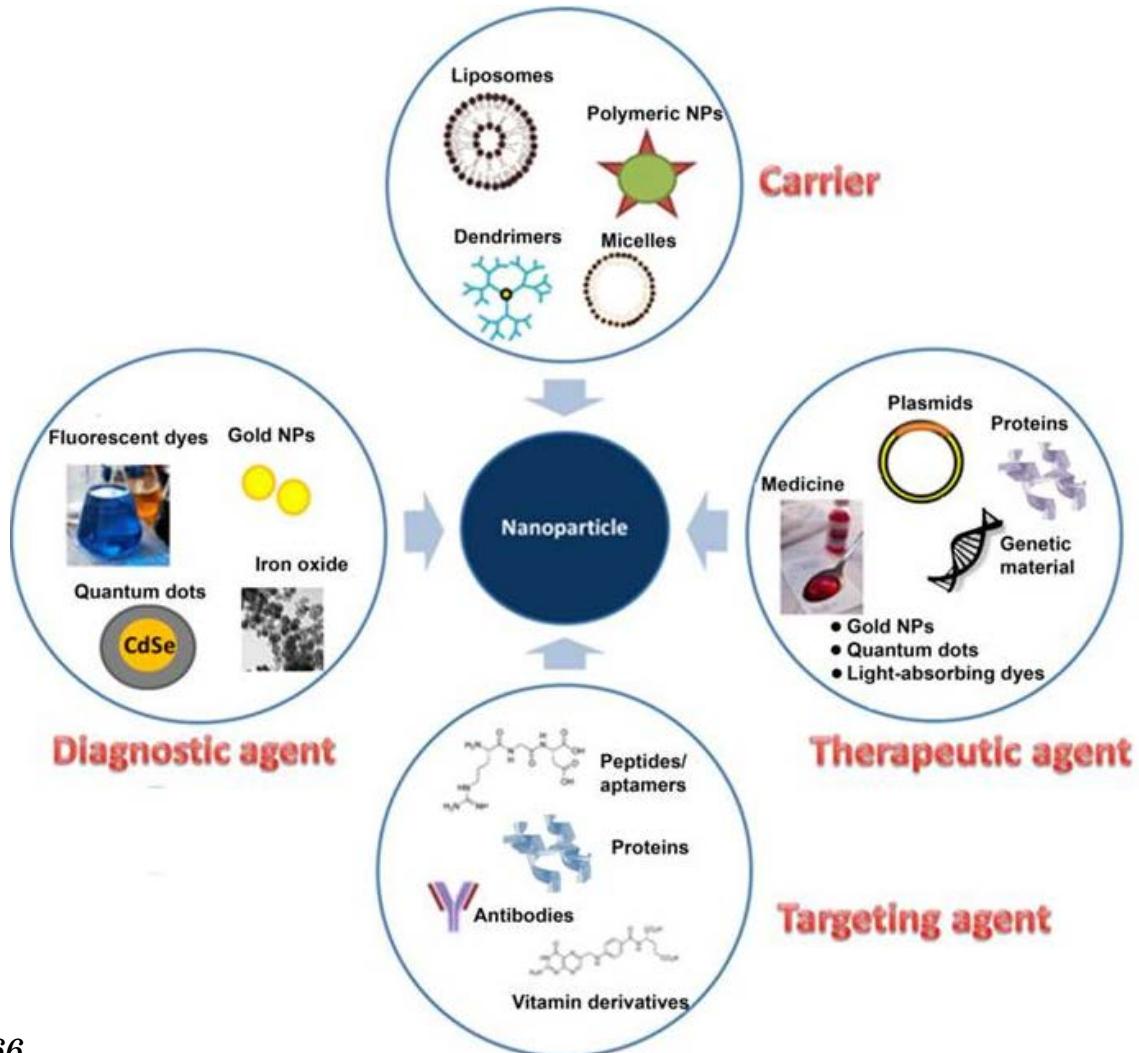
- Novel physical properties
- Increase stability and effect
- Reduce toxicity



Optimized drug delivery

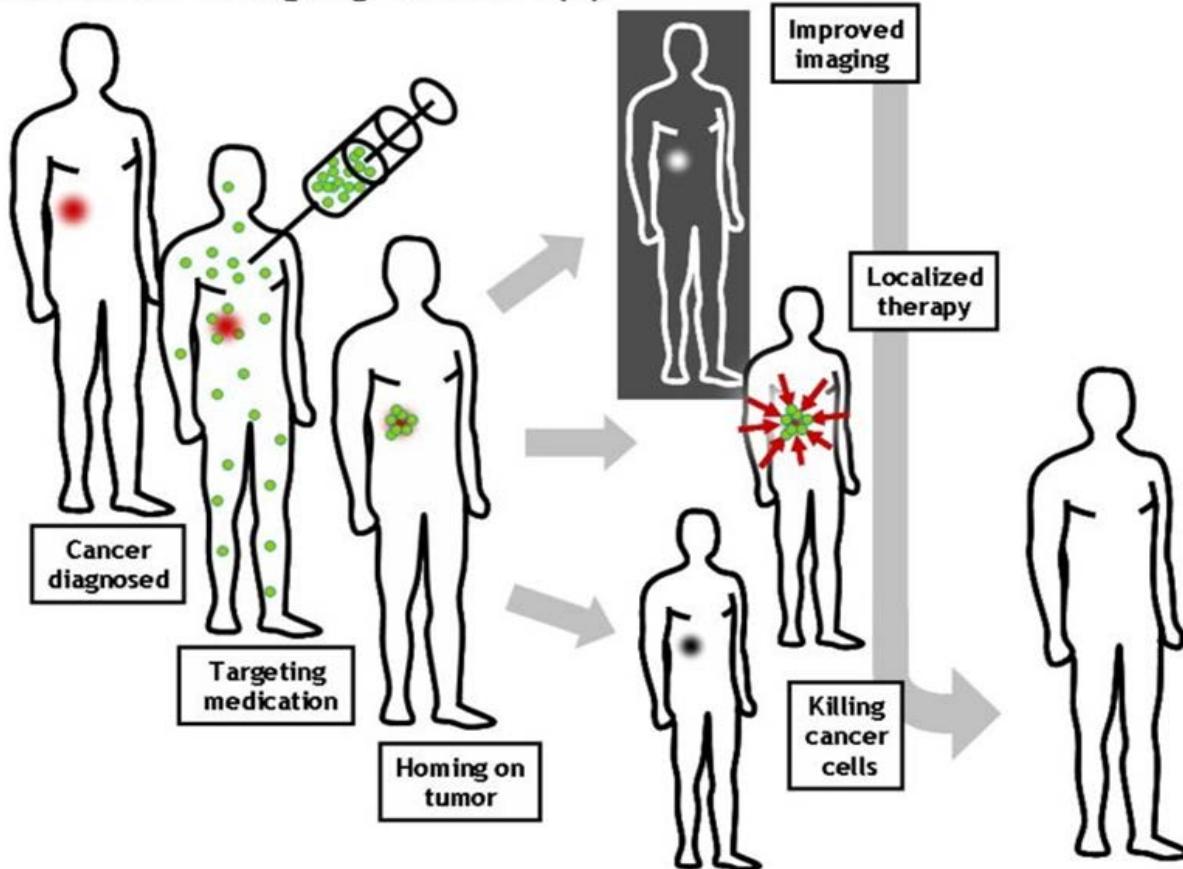
Different aspects of nanomedicine

Diagnostics - Therapeutics - Theranostics



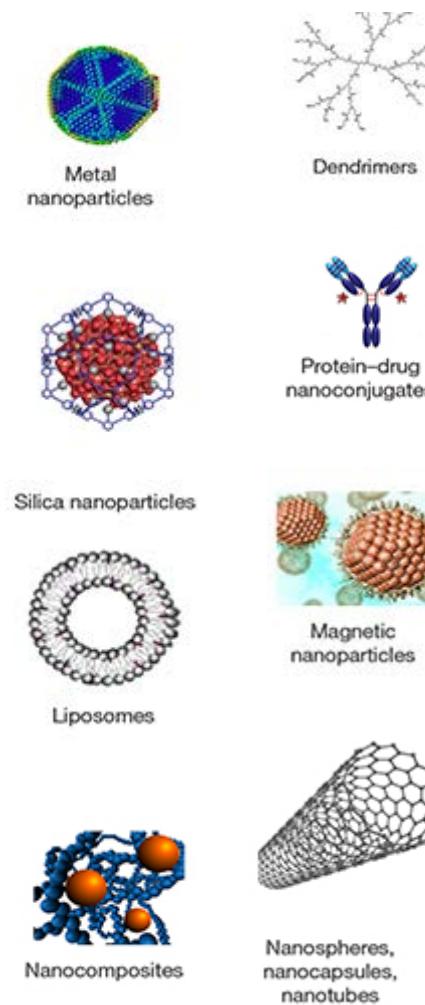
Uses of nanomedicine in oncology

Molecular imaging & therapy



Different types of nanoparticles for medical application

- Lipid-based
 - Liposomes
 - Other self-assembly systems
 - Solid lipid nanoparticles, Lipid nanocapsules
- Polymer-based
 - Hydrogel particles
 - Polymer particles
 - Polymer conjugates
 - Dendrimers
- Surfactant-based
 - Micellar solutions
 - Microemulsions
- Proteins/polypeptides
 - Often PEGylated
 - Protein conjugates
- Inorganic
 - Metal NPs
 - Mesoporous silica
 - Carbon-based (graphene, carbon nanotubes etc)
- Nanocrystals



Nanomedicine status – drugs on the market and in the pipeline

Examples of nanomedicines approved in the EU market

Nanomedicine class	Active substance/brand name	Pharmaceutical form	Therapeutic indications	Nanomedicine class	Active substance/brand name	Pharmaceutical form	Therapeutic indications	
Nanoparticles	<i>Nab-paclitaxel</i> Abraxane®	Powder for suspension for infusion	Breast neoplasms Carcinoma non-small-cell lung Pancreatic neoplasma	Nanoemulsions	<i>Cyclosporine</i> Sandimmun Neoral®	Capsule, soft	Solid organ, bone marrow transplantation Endogenous uveitis Nephrotic syndrome Rheumatoid arthritis Psoriasis Atopic dermatitis	
	<i>Yttrium-90 radiolabelled ibritumomab tiuxetan</i> Zevalin®	Solution for infusion	Follicular Lymphoma					
	<i>Glatiramer acetate</i> Copaxone®, Synthon®	Solution for injection	Multiple sclerosis					
Liposomes	<i>Doxorubicin hydrochloride</i> Caelyx®	Concentrate for solution for infusion	Breast neoplasms Multiple myeloma Ovarian neoplasms Kaposi's sarcoma Metastatic breast cancer	Nanocrystals	<i>Pegaspargase</i> Oncaspar®	Solution for injection/infusion.	Acute lymphoblastic leukemia	
	<i>Doxorubicin hydrochloride</i> Myocet®	Powder, dispersion and solvent for concentrate for dispersion for infusion	Fungal infection		<i>Paliperidone palmitate</i> Xeplion®		Schizophrenia	
	<i>Amphotericin B</i> AmBisome®	Powder for solution for infusion			<i>Olanzapine pamoate</i> Zypadhera®			
	<i>Daunorubicin</i> DaunoXome®	Concentrate for Solution for Infusion	Advanced HIV-related Kaposi's Sarcoma		<i>Aprepitant</i> Emend®		Nausea and vomiting	
	<i>Cytarabine</i> DepoCyt®	Suspension for injection	Lymphomatous meningitis		<i>Fenofibrate</i> Tricor®		Hiperlipidemia	
	<i>Mifamurtide</i> Mepact®	Powder for concentrate for dispersion for infusion	Osteosarcoma		<i>Lipanthyl®</i> Lipidi®		Graft rejection	
	<i>Morphine</i> DepoDur®	Suspension for injection	Pain		<i>Sirolimus</i> Rapamune®			
	<i>Verteporfin</i> Visudyne®	Powder for solution for infusion	Degenerative myopia, age-related macular degeneration	Polymer-protein conjugates	<i>Perginterferon alpha-2b</i> Pegintron®	Powder and solvent for solution for injection	Chronic hepatitis C	
	<i>Ferumoxytol</i> Rienso®	Solution for infusion	Iron deficiency anemia in adult patients with chronic kidney disease		<i>Perginterferon alpha-2a</i> Pegasys®			
	<i>Ferric carboxymaltose</i> Ferinject®	Solution for injection/infusion	Iron deficiency		<i>Pegfilgastrim</i> Neulasta®			
	<i>Iron(III) isomaltoside</i> Monofer®	Solution for injection/infusion.	Iron deficiency		<i>Methoxy polyethylene glycol-epoetin beta</i> Mircera®		Anemia associated with chronic kidney disease	
	<i>Iron(III)-hydroxide dextran complex</i> Ferrosat®	solution for infusion or injection	Iron deficiency		<i>Cetolizumab pegol</i> Cimzia™			
					<i>Pegvisomant</i> Somavert®		Rheumatoid arthritis	
							Acromegaly	

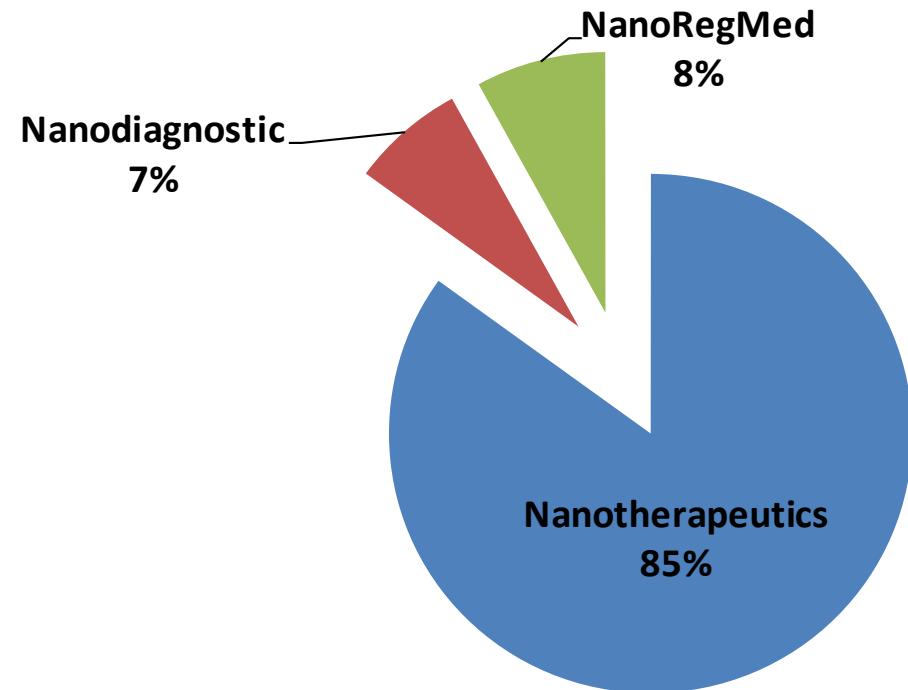
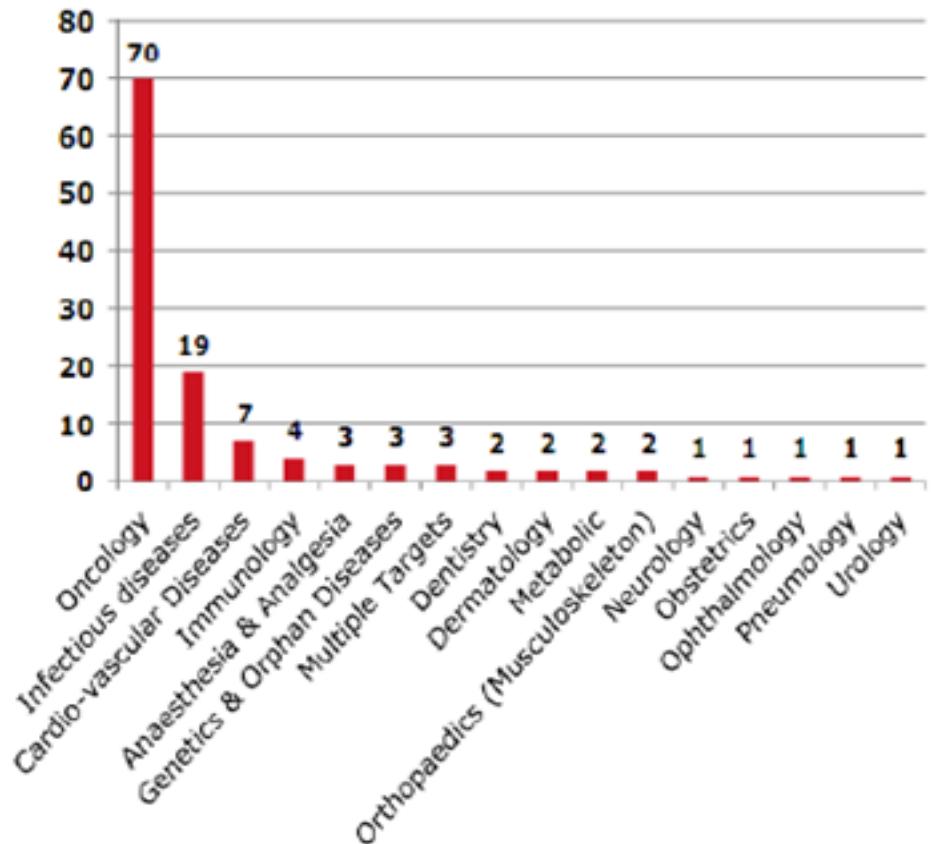
Nanomedicine status

In 2012:

122 pharmaceutical products + 25 devices in clinical development → 789 ongoing clinical trials

14% in clinical phase II-III

57% Oncology



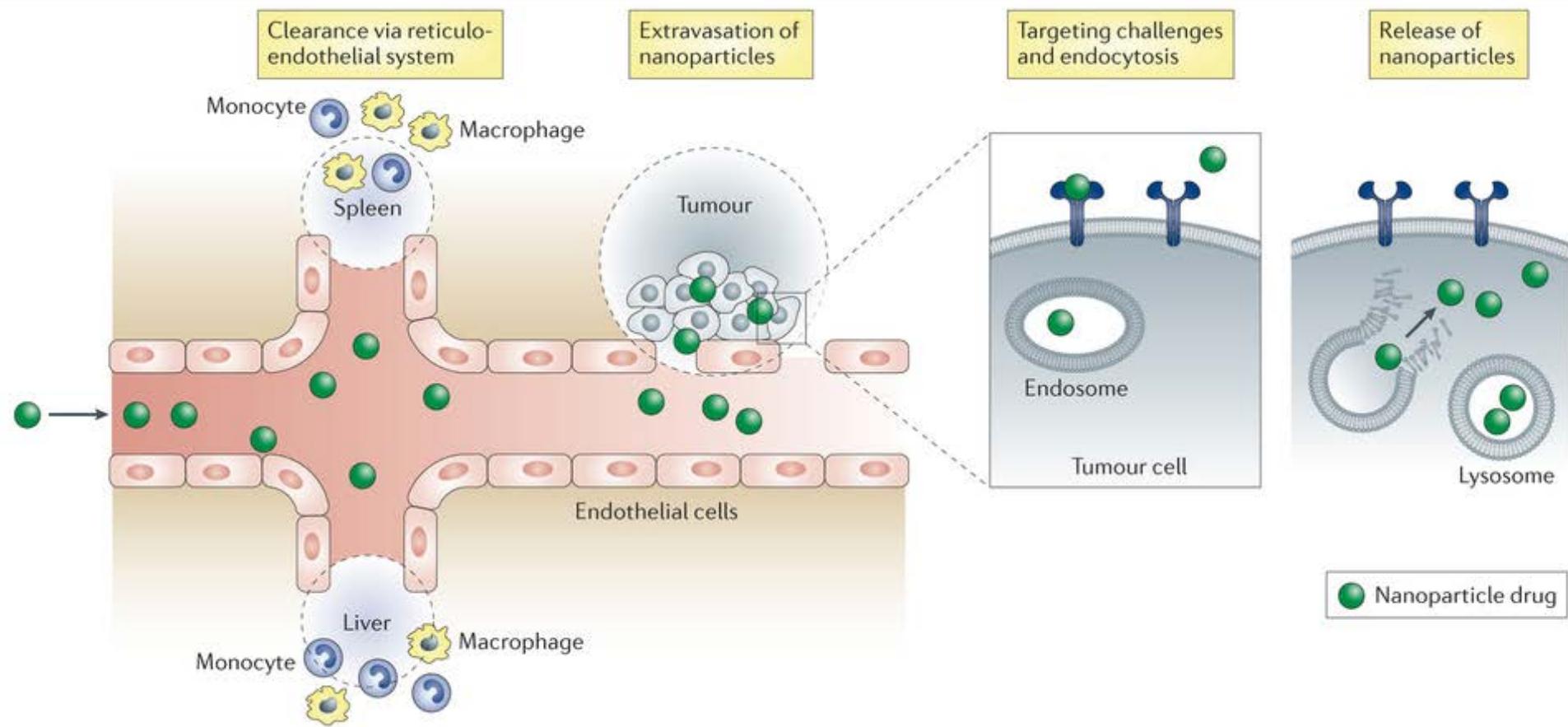
Among the 13 products in phase III:

- Oncology: 4
- Infectious diseases: 3
- Cardiovascular diseases: 3
- Immunology: 2
- Ophthalmology: 1

Nanomedicine - challenges

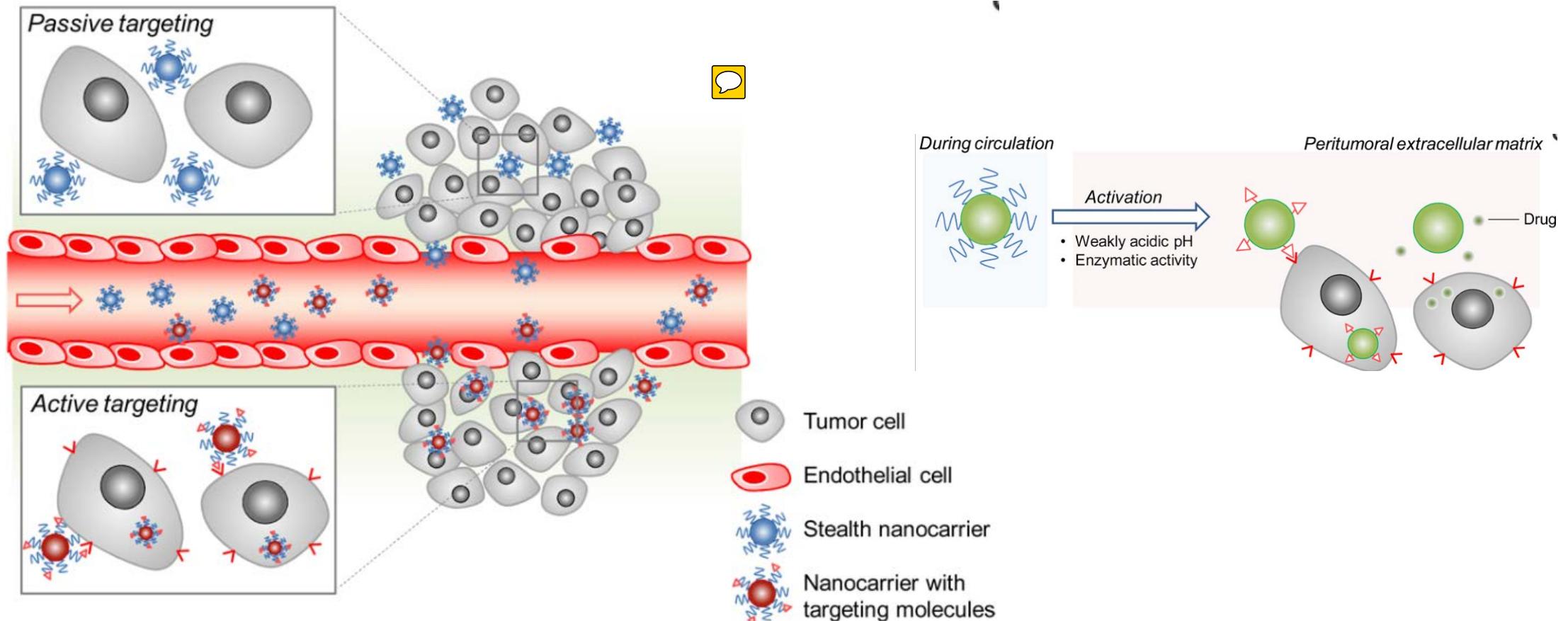
RES

Reticuloendothelial system/
Mononuclear phagocyte system



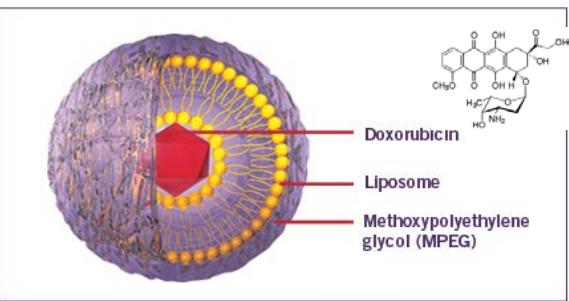
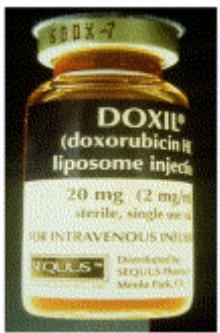
Nanomedicine – targeted drug delivery

EPR = Enhanced permeability and retention in target tissue (e.g. tumors)



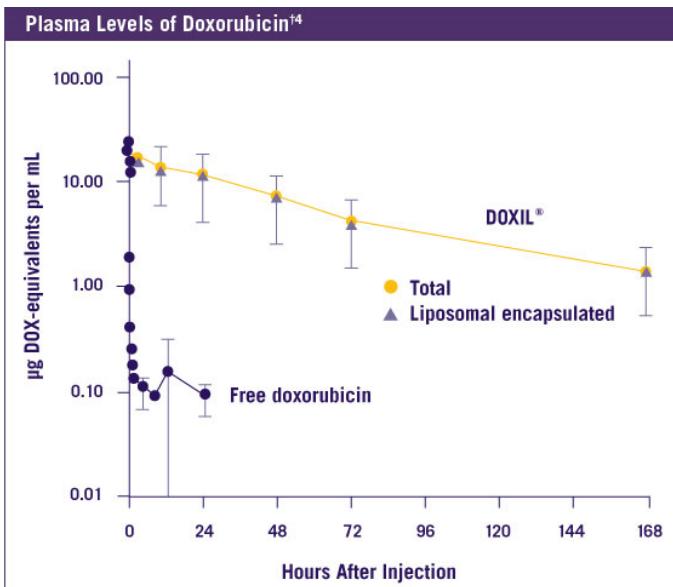
Commercial nanomedicine products - Liposomes

Doxil®
/Cealyx
1995



**Liposomes with
Doxorubicin
80–90 nm
Surface-grafted with PEG**
15,000 molecules payload

Kaposi's sarcoma (1995)
Ovarian cancer (1998)
Breast cancer (2003)

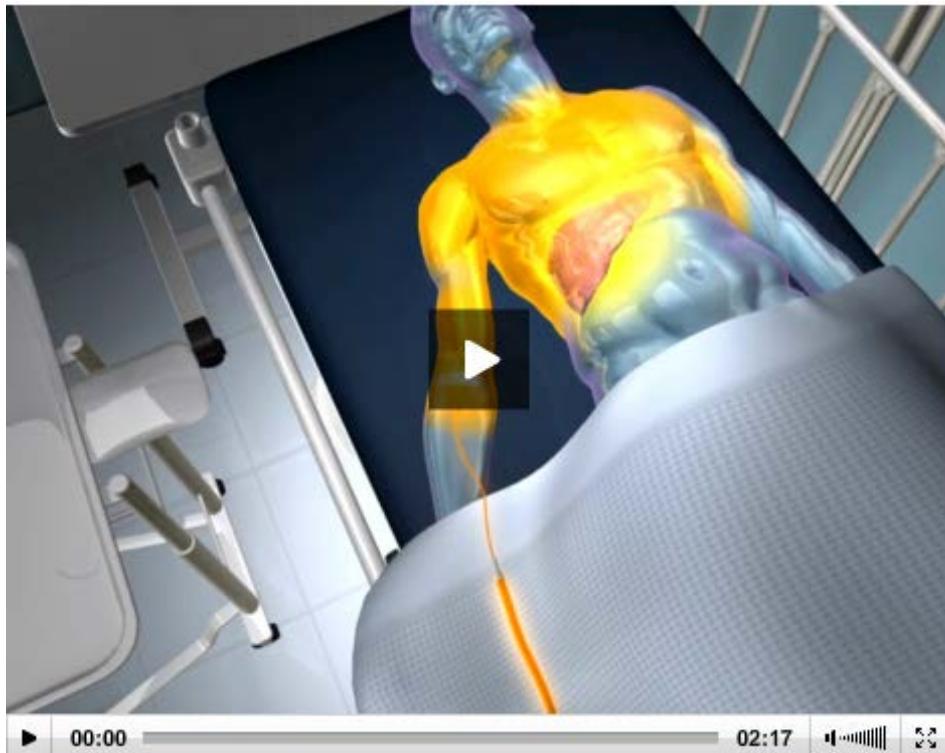


- 1) prolonged drug circulation time and avoidance of the RES
- 2) drastic decrease in the cardiotoxicity

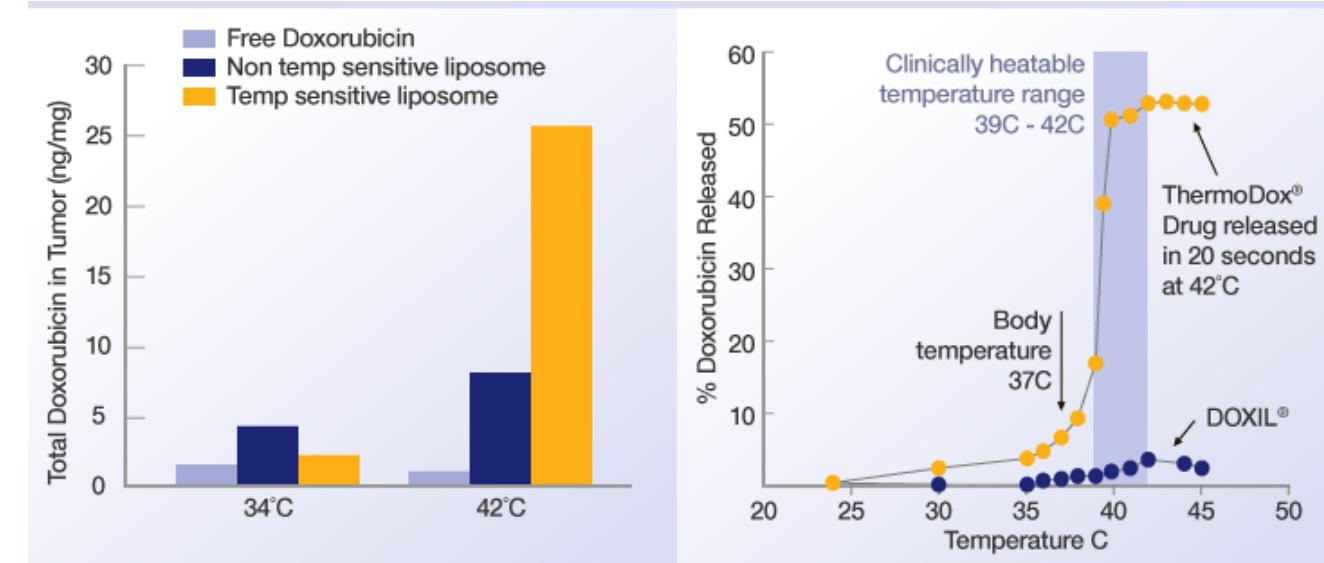
Related:
Myocet - without PEG- fast release
DaunoXome-Daunorubicin

Liposomes - late stage clinical development

ThermoDox® - heat activated liposomes, burst release



http://celsion.com/docs/technology_thermodox



FDA Approves Celsion's Study Of ThermoDox For Liver Cancer

March 2014 Now in Phase III

Delivers 25 times more doxorubicin into tumors

~550 patients
Patient enrollment complete in 2018

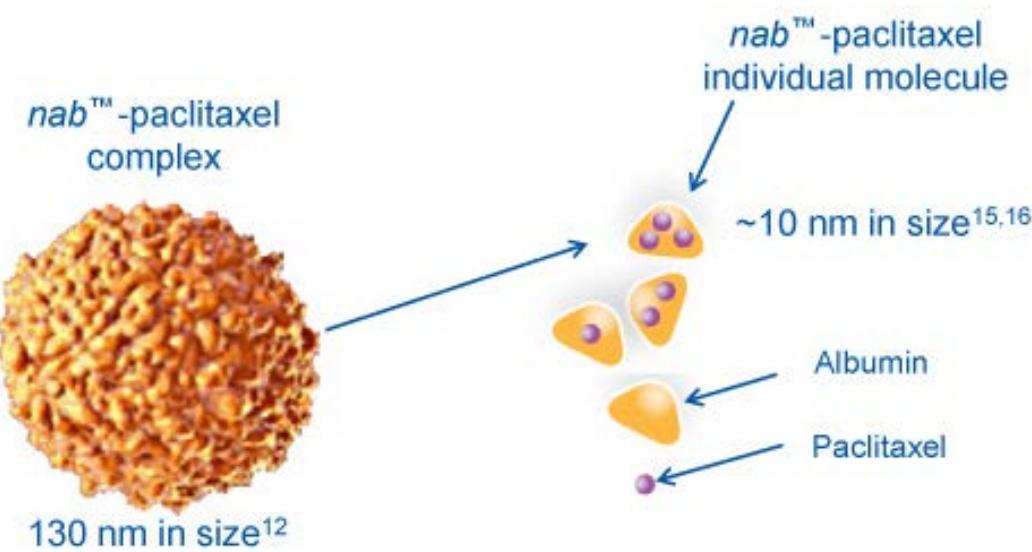
Commercial nanomedicine products – Protein nanoparticle

Abraxane®
2005



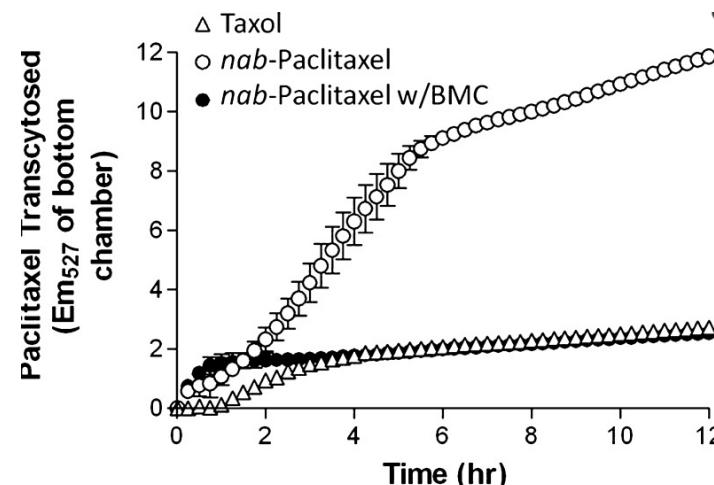
**human albumin and paclitaxel
130 nm**

Breast cancer
Lung cancer
Pancreatic cancer



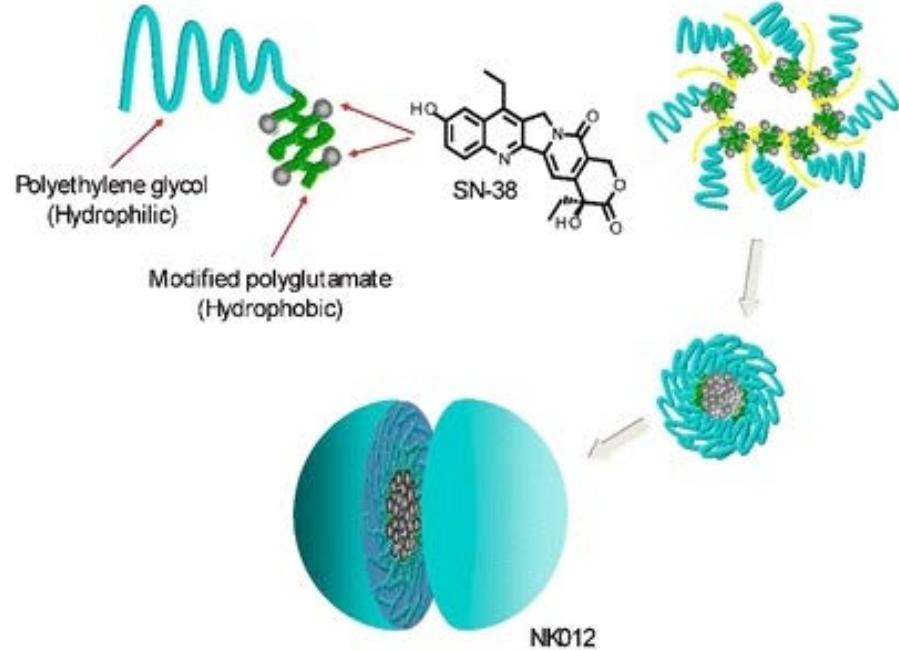
A single molecule of albumin can bind up to 6 or 7 molecules of paclitaxel^{¹¹}

- Poorly soluble drug
- Take advantage of natural albumin transport pathways to achieve more efficient binding to and moving across endothelial cell
- Reduced side-effects



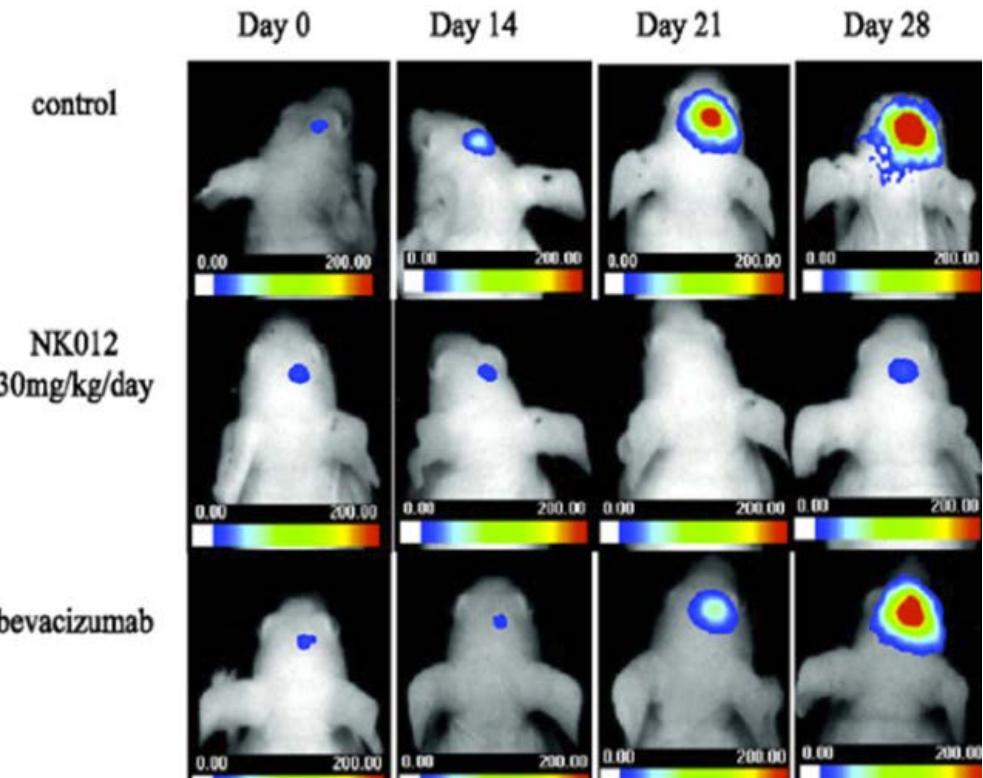
AAPS J. Jun 2012; 14(2): 282–295

Product in the pipeline - Polymeric Micelles



NK-012 Polymeric micelle of SN-38 Various cancers

American Association for Cancer Research



Kuronda et al. Clin Cancer Res. 2010, 16(2):521-9.

In Phase II trials

Nanocrystals

- Improve bioavailability of Class 2 molecules
- Improve rate of absorption
- Reduce Fed/Fasted variability
- Convenient and patient compliant dosage forms

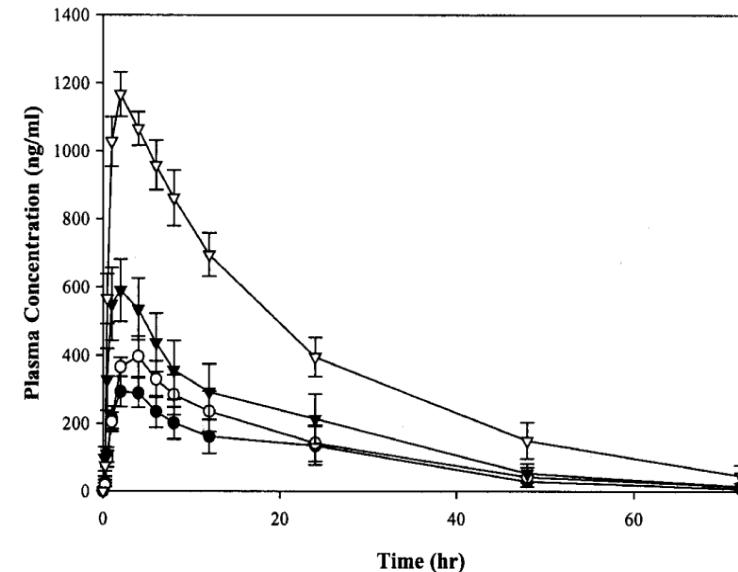
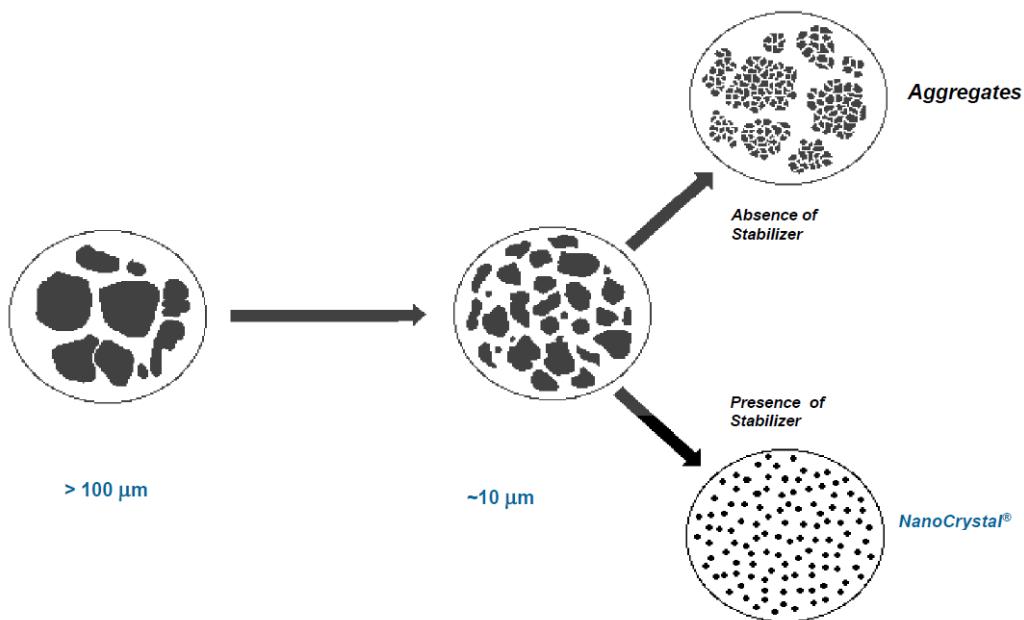


Fig. 3. Comparison of mean (\pm S.E.) plasma concentrations of MK-0869 following oral administrations in Beagle dogs ($N=5$) of suspensions made of conventional (●, 5.5 μ m), jet-milled (○, 1.8 μ m), wet-milled (▼, 0.48 μ m), and nano-milled (▽, 0.12 μ m) MK-0869 at a dose of 2 mg/kg under fasted conditions.

Intl. J. Pharm., 285, 2004, pp.135-146.

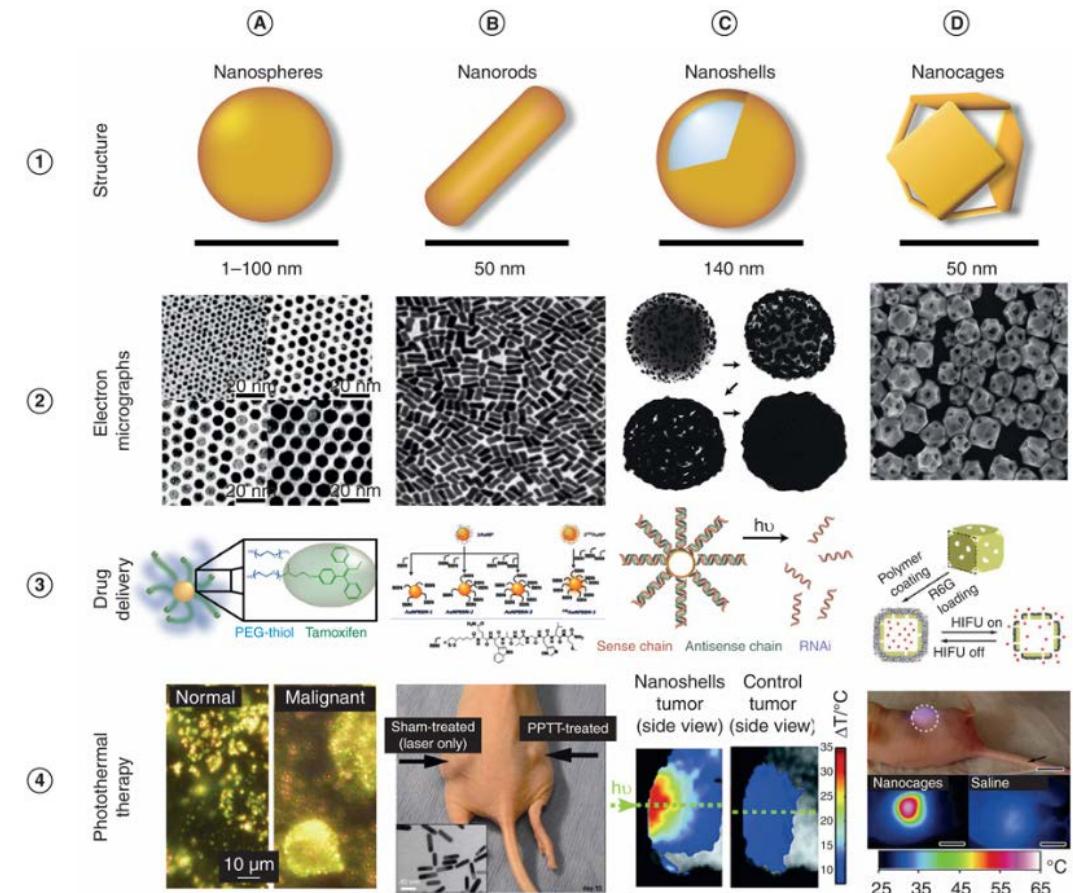
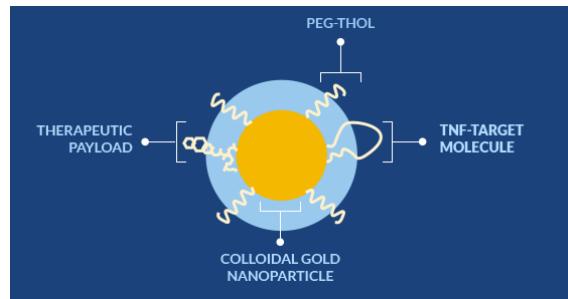


Rapamune
Immunosuppression

Gold Nanoparticles – in the pipeline

- Easily synthesized in large scale
- Functionalization
- Combination of physical, chemical, optical and electronic properties
- Highly multifunctional platform with which to image and diagnose diseases
- To selectively deliver therapeutic agent
- Enhanced drug pharmacokinetics/biodistribution

Aurimune nanomedicine platform (*CytImmune*)



Dreaden et al. Ther Deliv. 2012, 3(4): 457–478.

Phase I - PEGylated Au nanoparticles are well-tolerated in humans at therapeutically relevant dosages for TNF α .
Phase II – pancreatic cancer. Status?

The Future of Nanomedicine

Global nanomedicine market

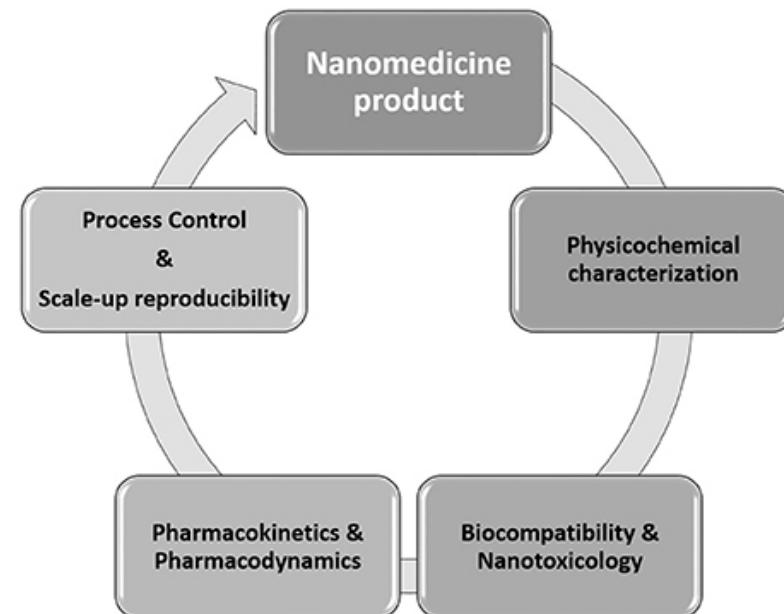
- The Global Nanomedicine Market is poised to grow at a CAGR of around 16.6% over the next decade to reach approximately \$1.3 trillion by 2025
- Nanomedicine: >10% of pharma sales

Source: <http://www.researchandmarkets.com/research/cd5j85/global>



Challenges

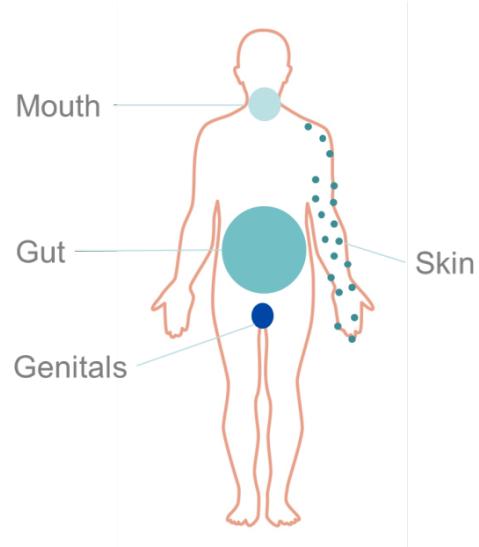
- **Nano-characterization**
 - Physical, chemical and biological evaluation
 - Synchronization with EMA (regulatory)
 - Mirror site to US/NCL
- **Manufacturing of clinical batches**
 - Scale up of manufacturing process
 - From lab to GMP unit
- **Regulatory issues**



Infectious diseases, antimicrobial resistance and nanomedicine



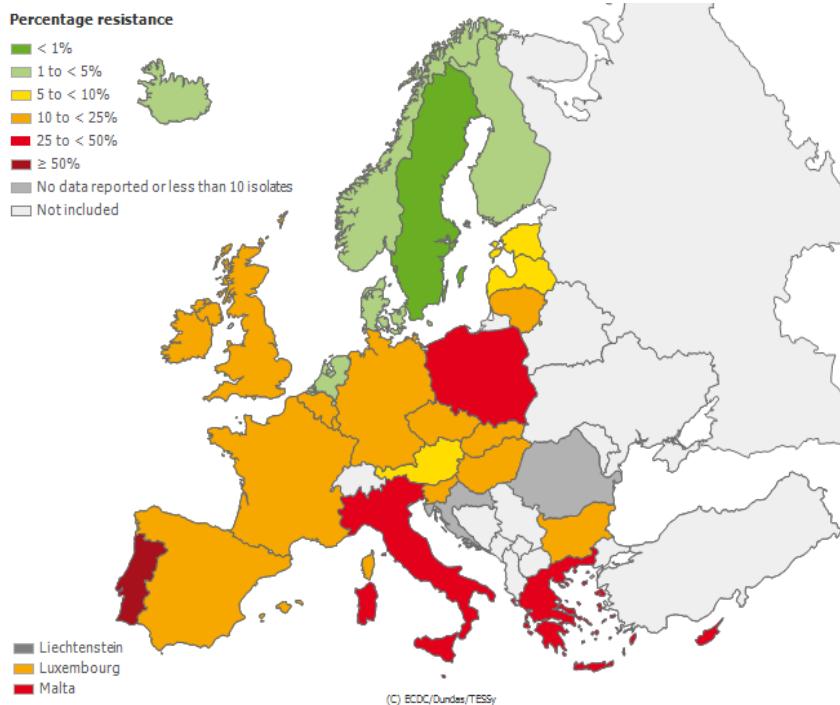
Bacteria and infectious diseases



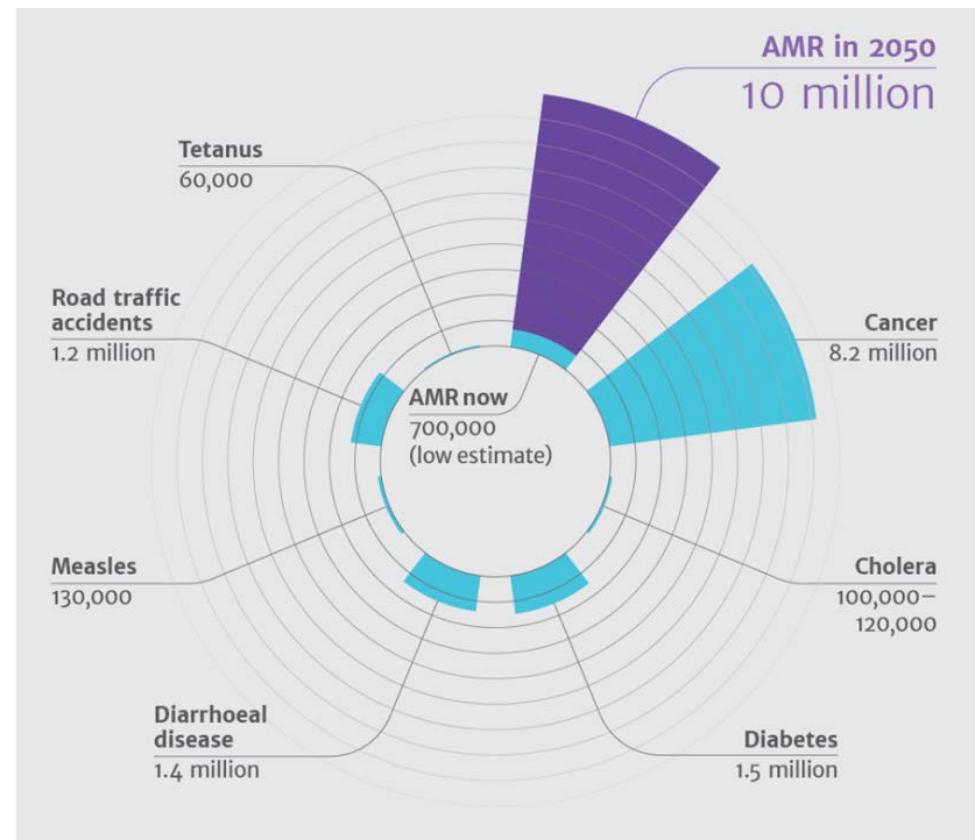
Antimicrobial resistance – a global threat

Infections today causes around 700 000 deaths per year globally (in combination with other diseases: 40% of all deaths)

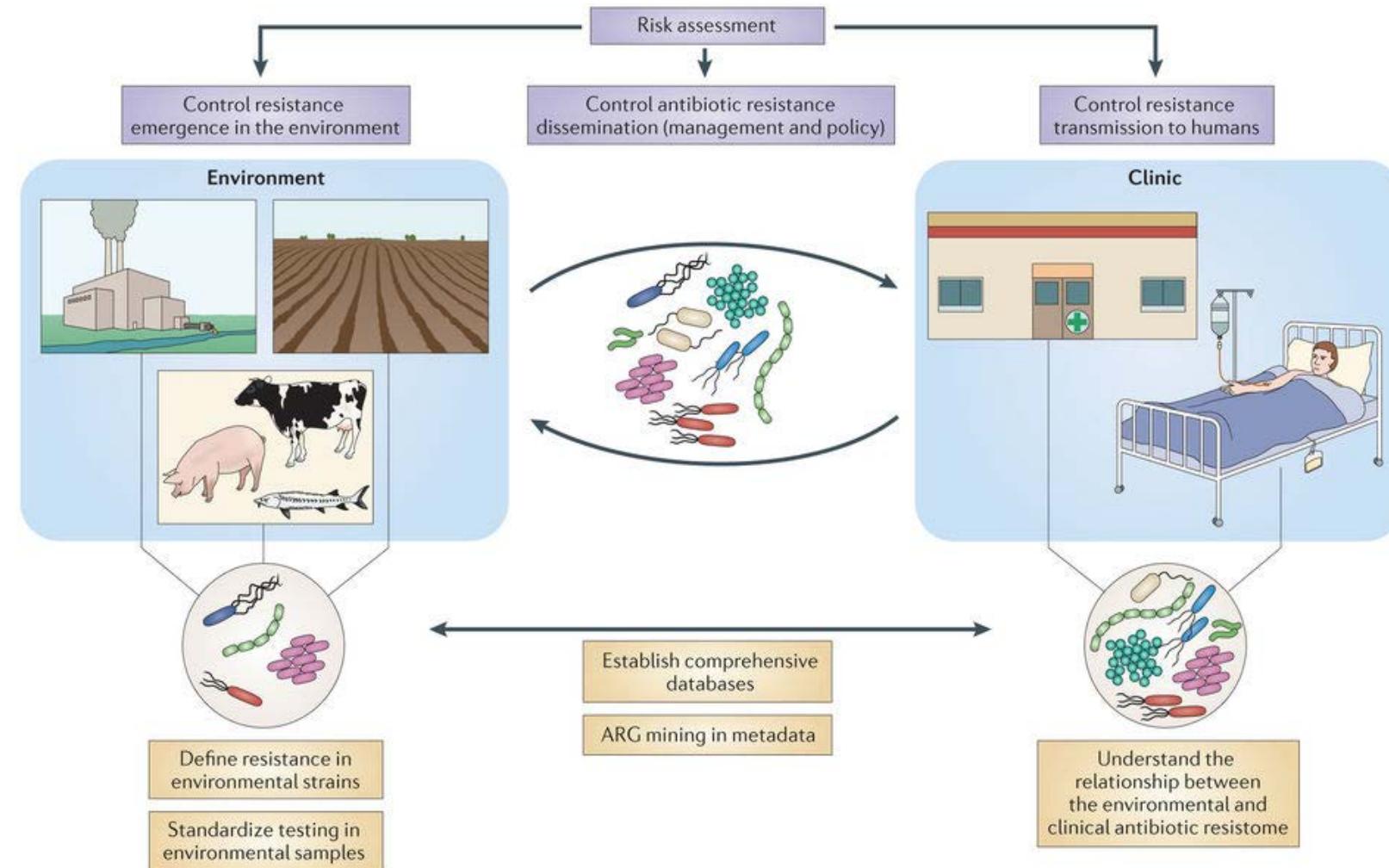
Methicillin resistant *S aureus* (MRSA)



If resistance development continues at the same pace as today - 10 million deaths per year in 2050



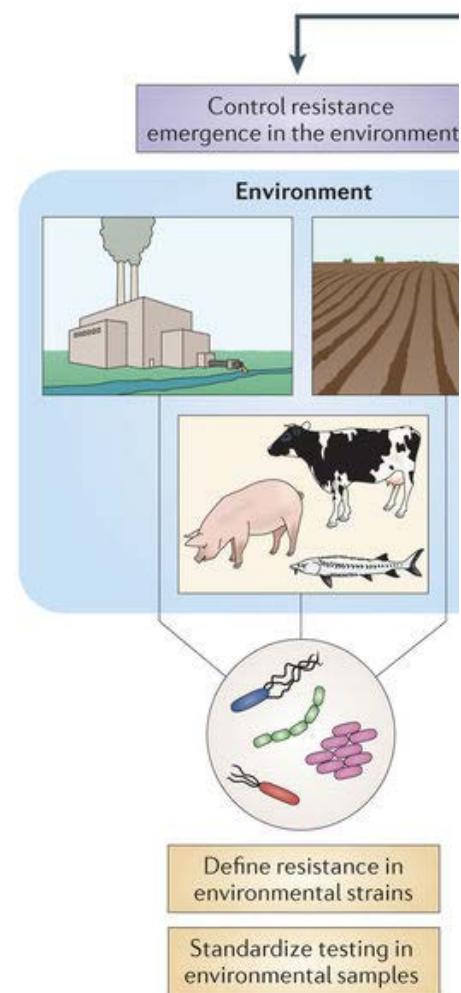
AMR in a One-health perspective



80% of the antibiotics in US used in agriculture

Resistant bacterial spread globally through people, water, climate changes

AMR in a One- Health Approach



Our time with
ANTIBIOTICS
is running out.

Antibiotics are in danger of losing their effectiveness due to misuse and overuse, and in many cases they aren't even needed.

Always seek the advice of a healthcare professional before taking antibiotics.



80% of the antibiotics in US used in agriculture

Resistant bacterial spread globally through people, water, climate changes

RISE Infection Management - in a "One Health" perspective

Prevention

Infection control



- Cleaning
- Hygiene
- Materials
- Technologies
- Vaccines

Treatment

Sustainable therapeutics



- Diagnostics
- Pharmaceuticals
- Reformulation
- New antimicrobials
- Clinical trial design



Monitoring

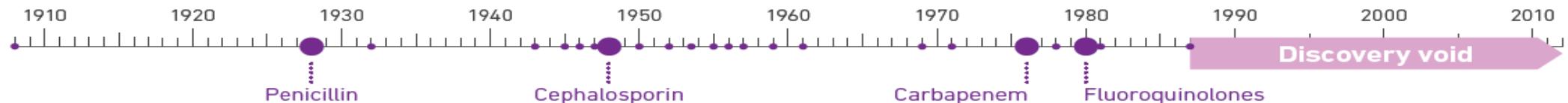
Knowledge transfer and surveillance



- Behavior
- Regulation
- Business Models
- Procurement
- Dissemination

New treatment strategies

Over the last 30 years, no major new types of antibiotics have been developed



Problems

- During several years, the large pharmaceutical companies have abandoned development of anti-infectives
- Diagnostics
- Clinical trials - high costs
- Regulatory

Needs

- New types of antibiotics for treatment of bacterial infections
- New types of antibiotics efficient against multidrug resistant bacteria
- More strategic use of existing antibiotics
- Restrict use of antibiotics
- Diagnostic tools

The current pipeline

There are only **37 antibiotics** in clinical development.



Historical data show that, generally, only

1 in 5

infectious disease drugs that enter Phase 1 trials will receive FDA approval.¹



Drugs can fail to receive approval for many reasons, including lack of effectiveness or safety concerns.

Alternatives to antibiotics - in the pipeline

Approach	Action	Spectrum	State of art
Antibodies	Bind and inactivation of pathogens	Gram+ and Gram-	Phase I-III
Probiotics	Therapeutic and profylactic	<i>C. Difficile</i> associated diarrhoea	Phase II-III
Lysins	Enzymes to destroy bacterial cell wall	Gram+	Phase I
Wild-type bacteriophages	Infect and kill bacteria	Gram+ and Gram-	Preclinical
Engineered bacteriophages	Infect and kill bacteria	Gram+ and Gram-	Preclinical
Immune stimulation	Enhance expression of innate immune response	Prevent or provide adjunct therapy for Gram+ and Gram- infections	Phase I-II
Vaccines	Reduce incidence of infection	Prevention, Gram + more than Gram-	Phase II-III
Antimicrobial peptides	Membrane disruptive, bactericidal and fast	Gram+ and Gram-	Preclinical-Phase II
Host defence peptides	Indirect antimicrobial effect	Gram+ and Gram-	Preclinical
Antibiofilm peptides	Specifically inhibit biofilm formation	Gram+ and Gram-	Preclinical

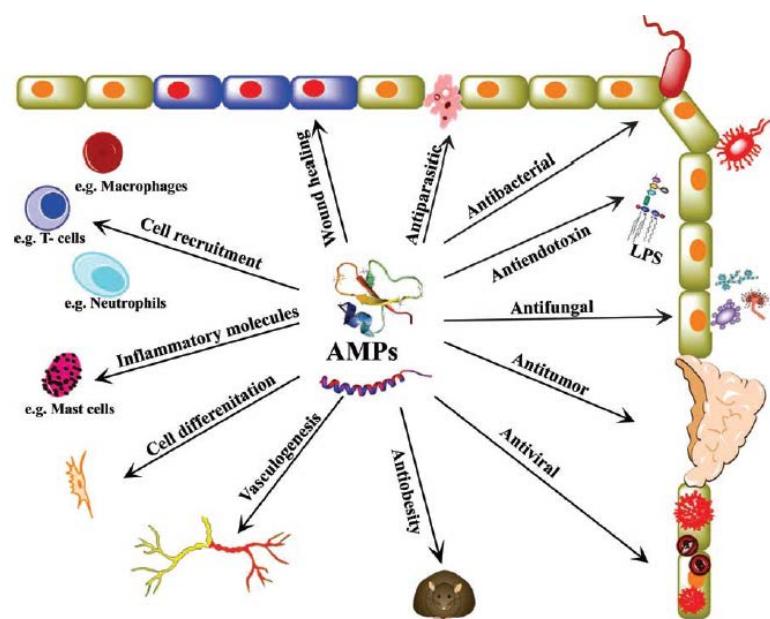


Antimicrobial peptides (AMPs) – an alternative approach

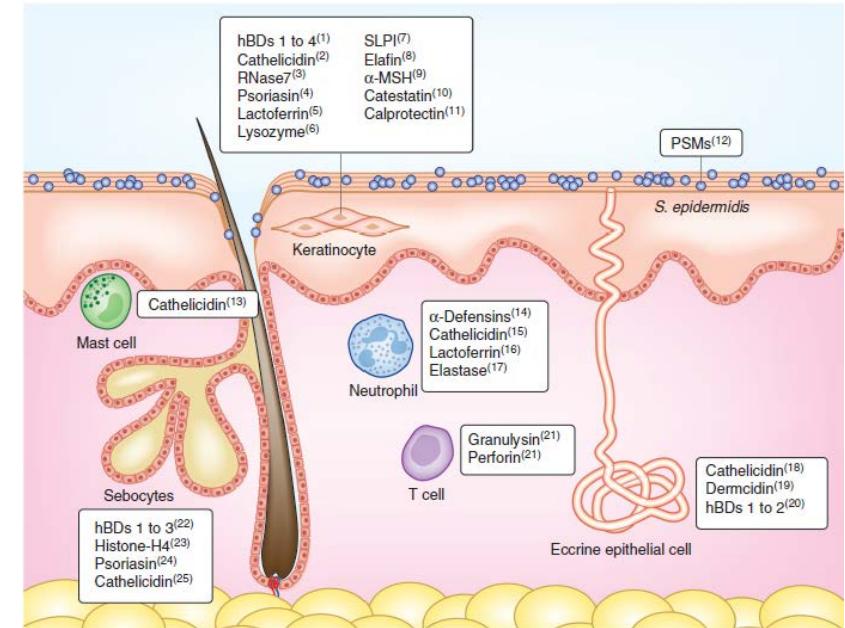
Around 2400 AMPs identified today

<http://aps.unmc.edu/AP/main.php>

- Present in plants, insects , animals and humans
- Part of the host defense system
- High concentrations in skin, airways, mucosa
- Expressed in response to pathogens
- Evolutionary well-preserved



Critical Reviews in Biotechnology, 2012; 32, 143



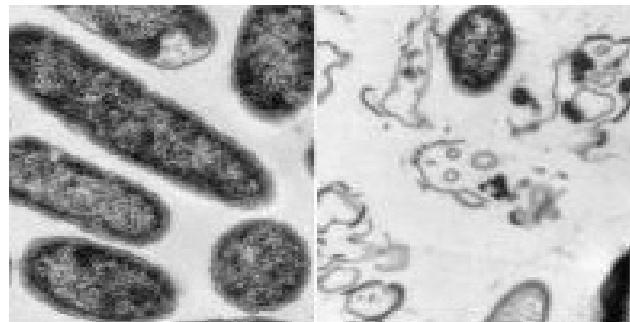
AMPs in skin

Journal of Investigative Dermatology, 2012, 132, 887

General properties

- 10-40 amino acids
- Cationic
- Amphiphilic

Antimicrobial peptides – Mechanism of action

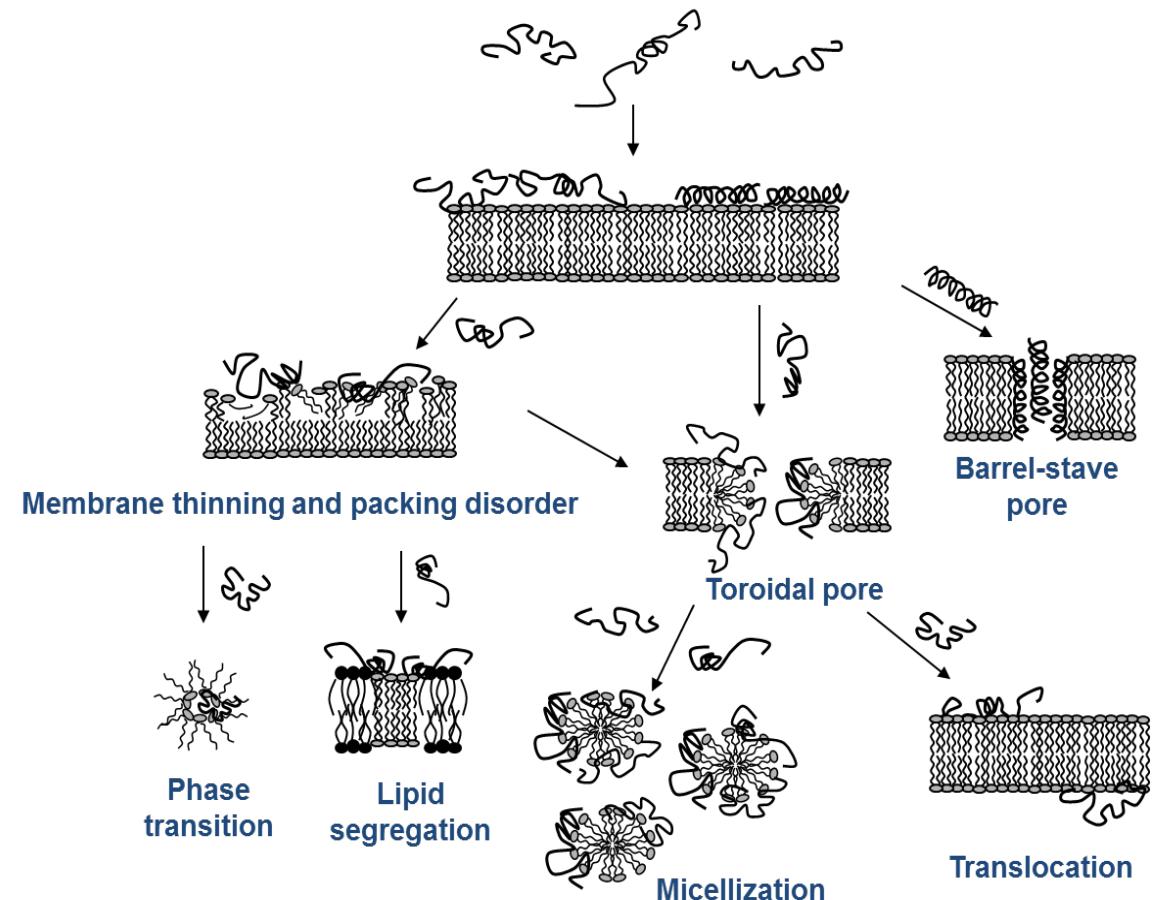
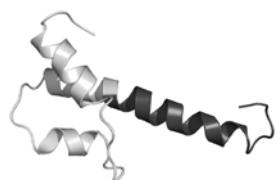


J Biol Chem, 2005, 280, 34832

- Fast and non-specific mechanism of action
- Bacteria not as prone to develop high level resistance

Efficiency and MoA influenced by

- Size
- Conformation
- Net charge
- Charge distribution
- Hydrophobicity



Ringstad, Uppsala University thesis, 2009

AMPs in drug delivery

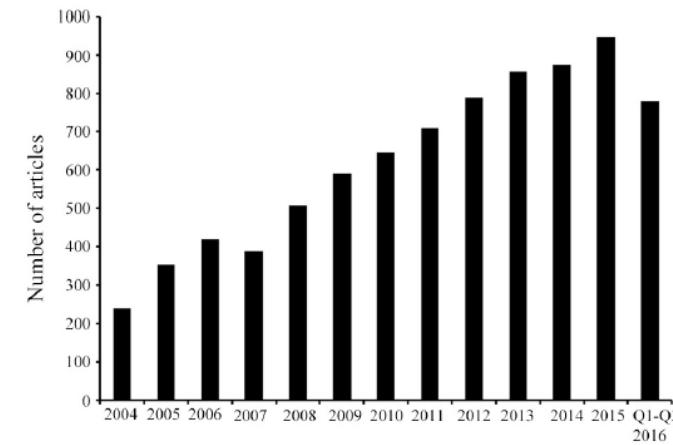
AMPs in clinical trials but few products on the market



Problems with:

- Stability (chemical and proteolytic)
- Not efficient enough
- Toxicity issues
- High cost

Published research on AMPs 2004-September 2016



Selected AMPs in clinical phase of development

AMP	Description	Phase	Indication	Administration	Clinical trial identifier if available
Pexiganan (MSI-78)	Analog of magainin (skin of African clawed frog)	Phase III	Infected diabetic foot ulcers	Topical cream	NCT00563394, NCT00563433
Omagigan	Derived from indolicidin (bovine)	Phase IV/III	Catheter infections and rosacea	Topical gel	NCT00231153, NCT01784133
Lytixar (LTX-100)	Synthetic antimicrobial peptidomimetic	Phase I/II	Uncomplicated Gram-positive skin infections, impetigo, and nasal colonization with <i>S. aureus</i>	Topical hydrogel	NCT01223222, NCT01803035, NCT01158235
hLF1-11	Derived from lactoferrin (human)	Phase I/II	Bacteremia and fungal infections in immunocompromised haematopoietic stem cell transplant recipients	Intravenous treatment (in saline)	NCT00509938
Novokatin (NP-213)	Derived from defensins (bovine)	Phase II	Oncornycomycetous fungal nail infection	Topical brush-on-treatment	
OTX-023	Oral cyclic cationic defensin (LL-37)	Phase I/II	Vaginal candidiasis	Vaginal gel	
PXL01	Derived from lactoferrin (human)	Phase II	Prevention of post-surgical adhesion formation in hand surgery	Polyvinyl alcohol-based solution for administration in the wound bed	NCT01022242
Isofuguan (IP-10)	Derived from interleukin-8 (granulocyte leukocytes)	Phase III	Oral mucositis in patients receiving radiotherapy for head and neck malignancy	Hyaluronic acid-based hydrogel for administration at the surgical site	
PAC-113	Derived from histatin 3 (human saliva)	Phase II	Oral candidiasis in HIV seropositive patients	Oral solution	NCT00022373
				Mouthrinse	NCT00659971

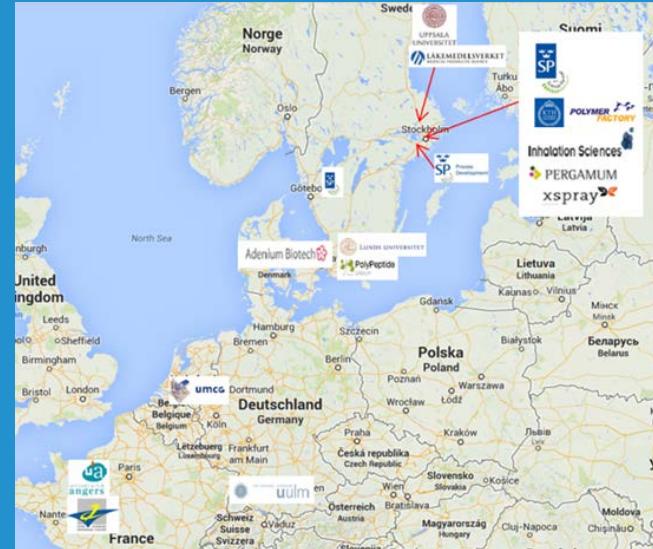
Mahlapuu et al. *Front Cell Inf Microbiol.* 2016, 6:194

Formulation

FORMAMP project



<https://www.youtube.com/watch?v=3Lmt8w5UyX0>



- Duration: 2013-2017, Budget: 10.5 MEuro
- EU contribution: 8 MEuro
- 16 partners from 5 countries
- www.formampproject.com



FORMAMP

Innovative nanoformulation of antimicrobial peptides

Vision: To reduce the alarming progress of multidrug-resistant bacteria

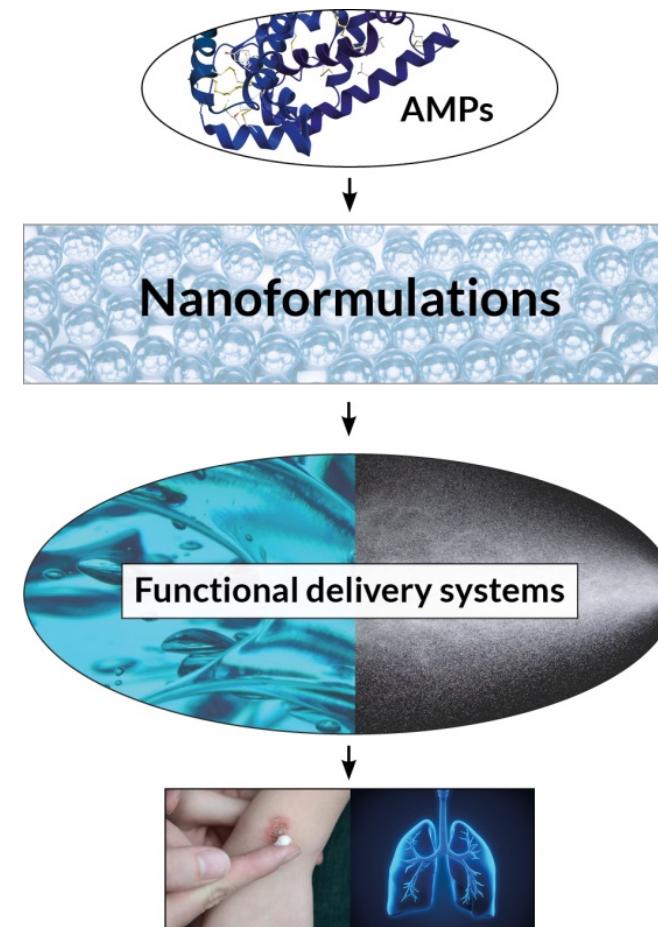
Mission: To develop new sustainable strategies for treatment of infectious diseases

Nanoformulation of AMPs to obtain:

- Improved chemical and proteolytic stability
- Triggered and controlled release
- Improved functionality and efficient combination therapies

Local delivery to:

- Reduce exposure
- Avoid side-effect
- Increase compliance



Targeted indications

- MRSA in wound infections (biofilms)
- Tuberculosis
- Cystic fibrosis (biofilm)

Functional delivery systems

- Topical gel or spray
- Pulmonary aerosol

Process development, scale-up and regulatory strategies

Skin and soft tissue infections - The Medical Need

- Skin indications associated with infections: Chronic wounds, burn wounds, atopic dermatitis, eczema, surgical site infections, secondary infected traumatic lesions
- Sub-groups of patients suffer from frequently recurring infections, e.g. secondary infections in dermatitis
- There is a major need for safe and effective antimicrobial products that can be used in for long term treatment including pediatric populations
- Local treatment is preferred in order to avoid systemic exposure and undesired side-effects
- Currently all major classes of topical antibiotics have some extent of emerging antibiotics resistance → **There are no antimicrobial product for use in SSTI that is compatible with long-term use without risk of resistance development**

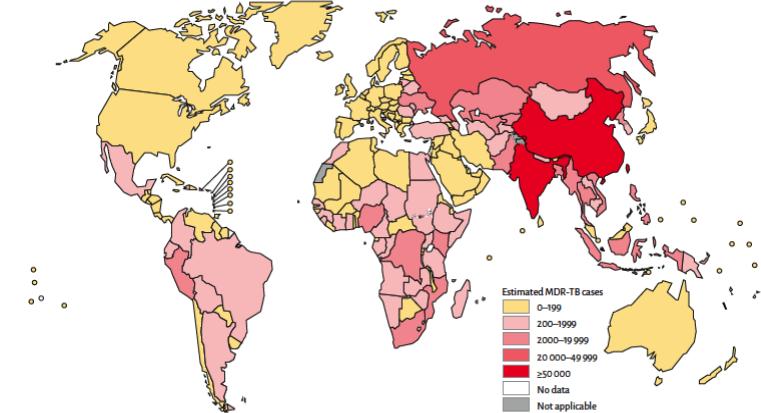


Tuberculosis – a global challenge

- Multidrug resistant tuberculosis is spreading
- Disease is located mainly to the lungs, but can be manifested in other sites (skeleton, ...)
- Spreads through air-borne bacilli when granuloma containing the *M. tuberculosis* are ruptured in the sick patient
- Only 10-15% of carriers develop the illness – of these up to 50% die if left untreated
- Treatment requires >12 months daily antibiotics, with different combinations of drugs– poor compliance is a severe challenge for successful outcome and continued resistance development
- Local delivery of antimicrobial peptides has the potential to improve treatment outcome and even enable eradication of the disease**

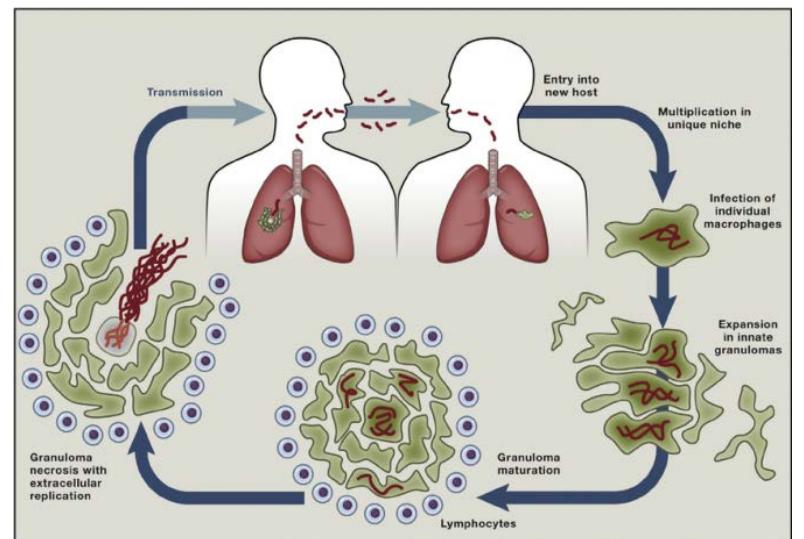
Number of MDR-TB among notified TB-cases

Number of MDR-TB cases estimated to occur among notified pulmonary TB cases, 2014



WHO report, 2015

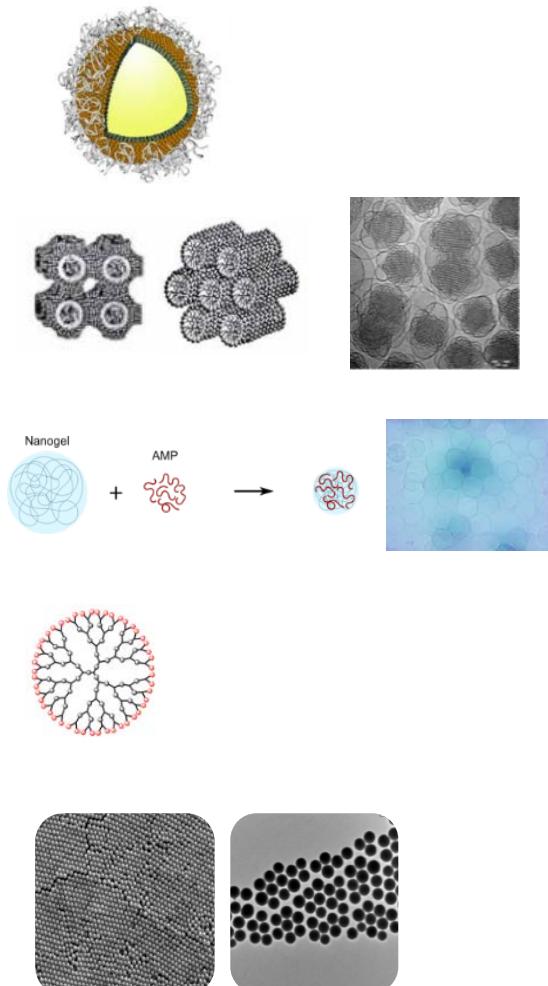
Pathogenic Life Cycle of *M. tuberculosis*



Cambier et al, 2014, Cell 159:1497

FORMAMP Nanocarriers

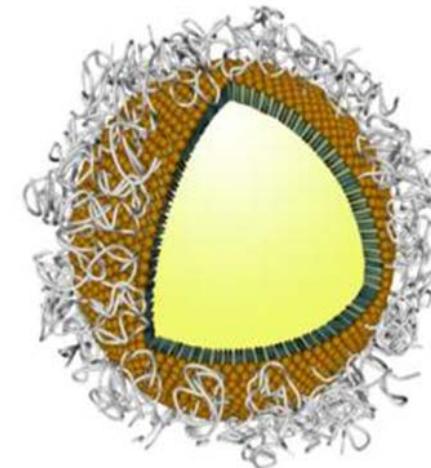
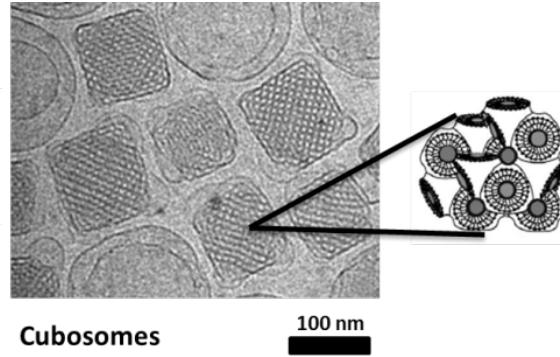
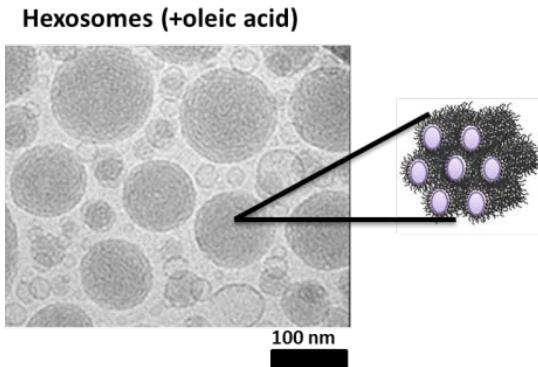
Formulation Strategy	Advantages
Lipidic nanocapsules (LNCs)	<ul style="list-style-type: none">Targeting to different organsSuitable for a large range of moleculesEasy to produce/scale upBiocompatible/biodegradable
Lipid self-assembly systems	<ul style="list-style-type: none">Suitable for mucosal and pulmonary deliveryBiocompatible/biodegradableNanoparticles and macroscopic structuresEasy to produce using conventional techniques
Microgels	<ul style="list-style-type: none">Hydrophilic, prevent aggregation of peptidesHigh loading capacity of AMPsStimuli-responsive release
Dendrimers	<ul style="list-style-type: none">Well-definedPrecise control of size, composition and structureLoading capacity can be monitoredBiocompatibleControlled degradation/release feasible
Mesoporous silica nanoparticles (MSNs)	<ul style="list-style-type: none">High loading capacityTargeting to different organsParticle size and shape controlEasy surface functionalizationRelease mechanisms



Project results

- AMPs can be formulated in all nanocarriers investigated with maintained/increased effect and low toxicity
- Encapsulation efficiency can be controlled through design of nanocarriers and peptide loading strategy
- Encapsulation can protect AMPs from proteolytic degradation
- Nanocarriers can enhance biofilm penetration and degradation
- One completely new type of carrier material has been developed

Lipid-based nanocarriers



Oil
Phospholipid
PEGylated surfactant

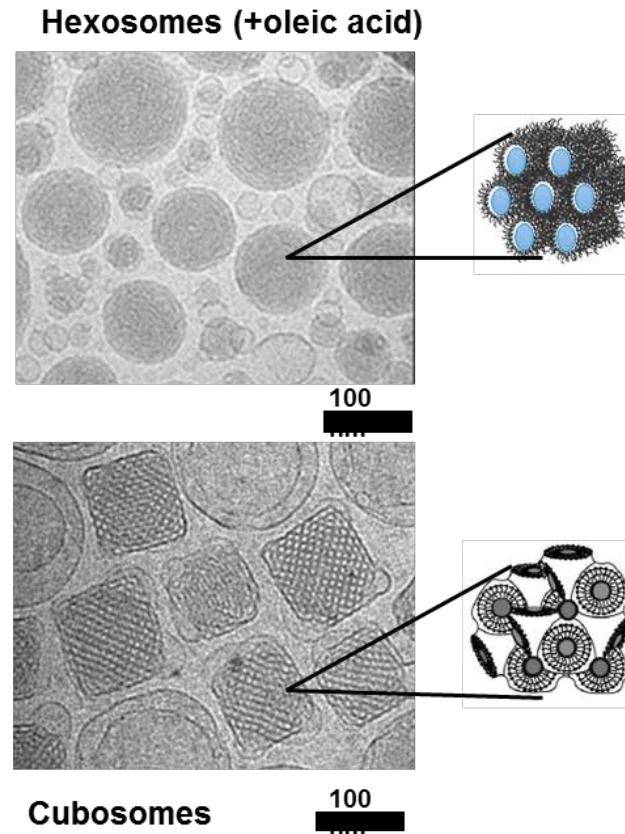
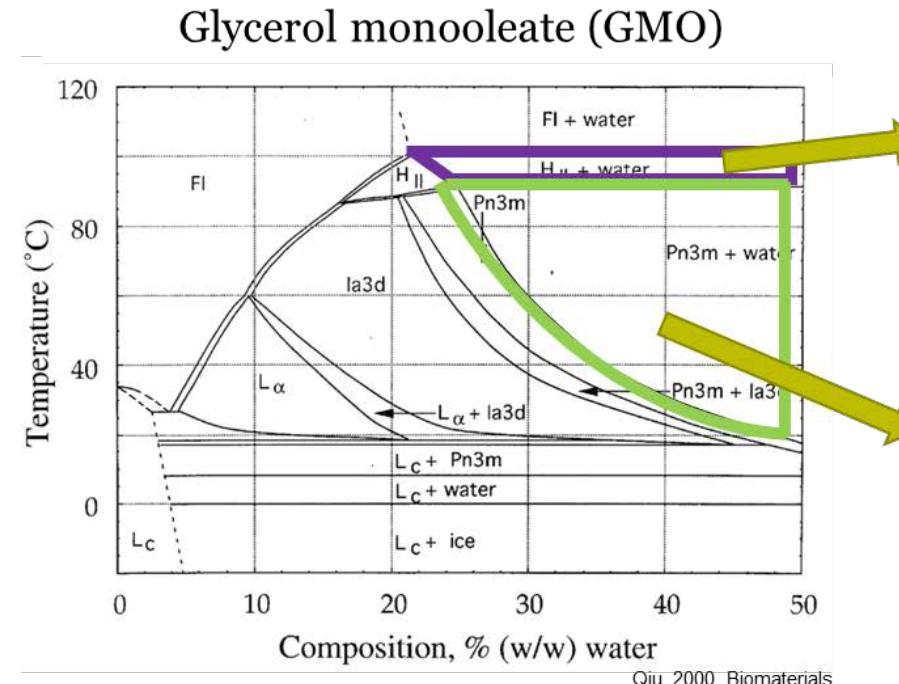
Liquid crystalline nanoparticles (LCNPs)

- Suitable for mucosal and pulmonary delivery
- Biocompatible/biodegradable
- Nanoparticles and macroscopic structures
- Easy to produce using conventional techniques

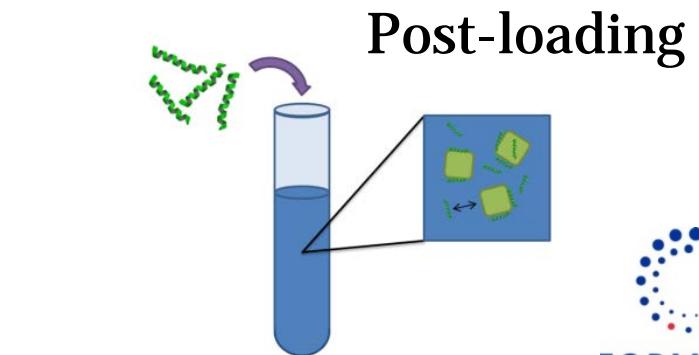
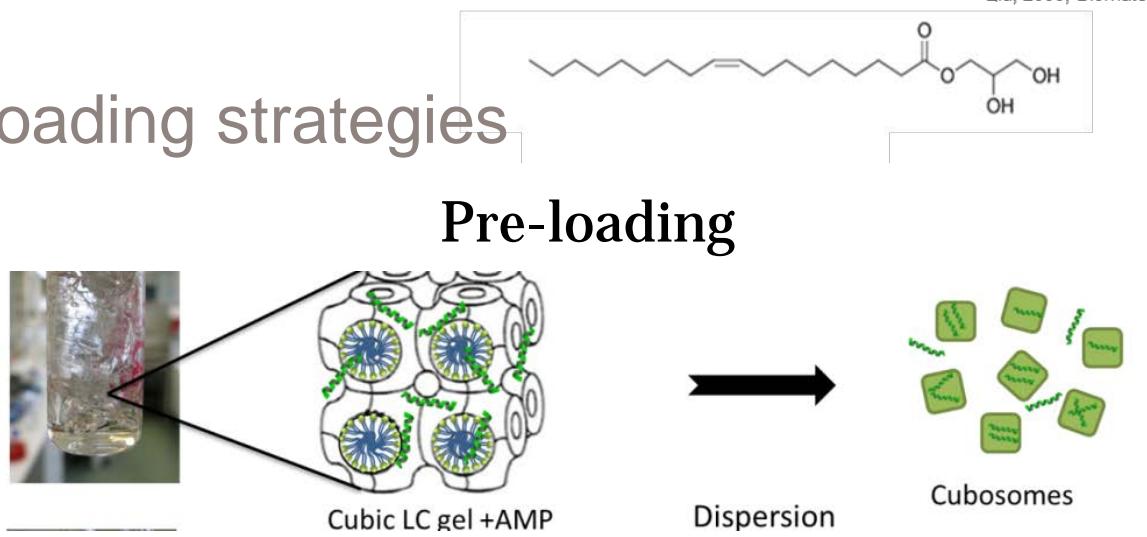
Lipidic nanocapsules (LNCs)

- Targeting to different organs
- Suitable for a large range of molecules
- Easy to produce/scale up
- Biocompatible/biodegradable

Liquid crystalline nanoparticles (LCNPs)

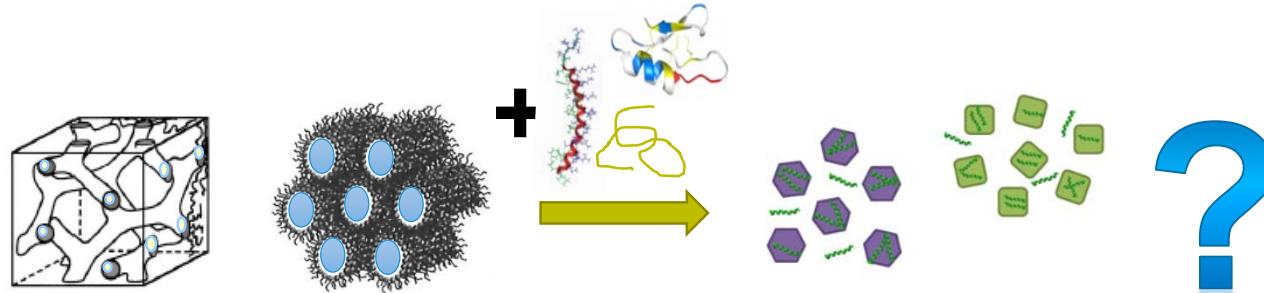


Loading strategies

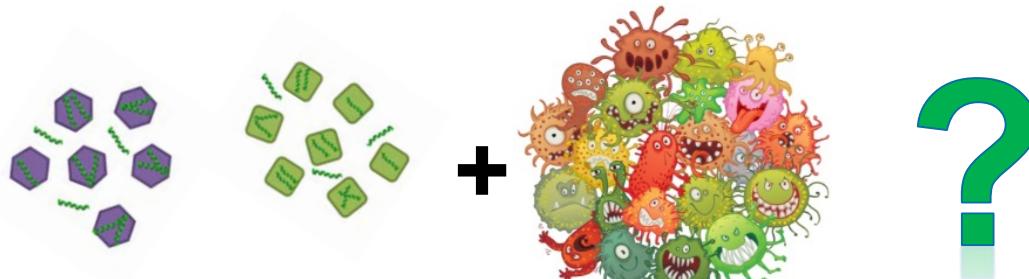


Outline of PhD project – Liquid crystalline nanoparticles

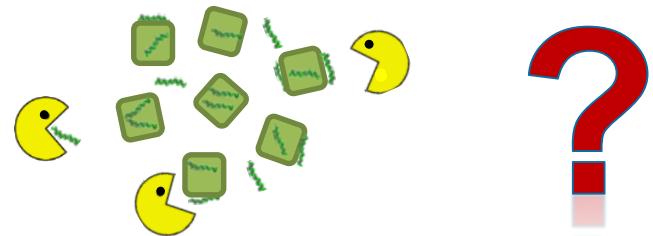
1.



2.

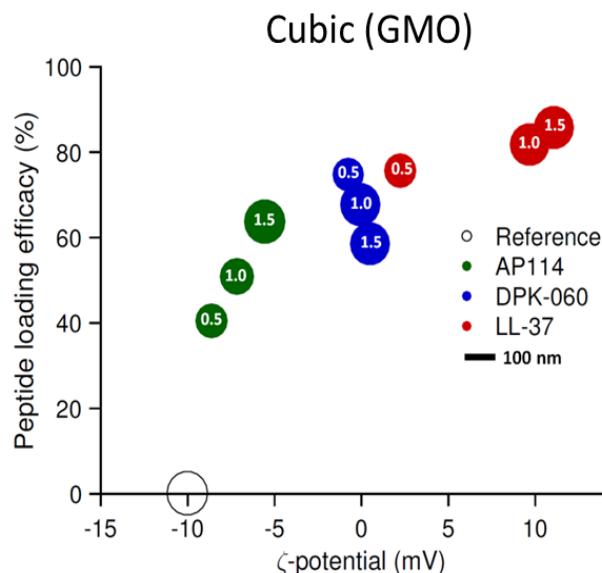


3.

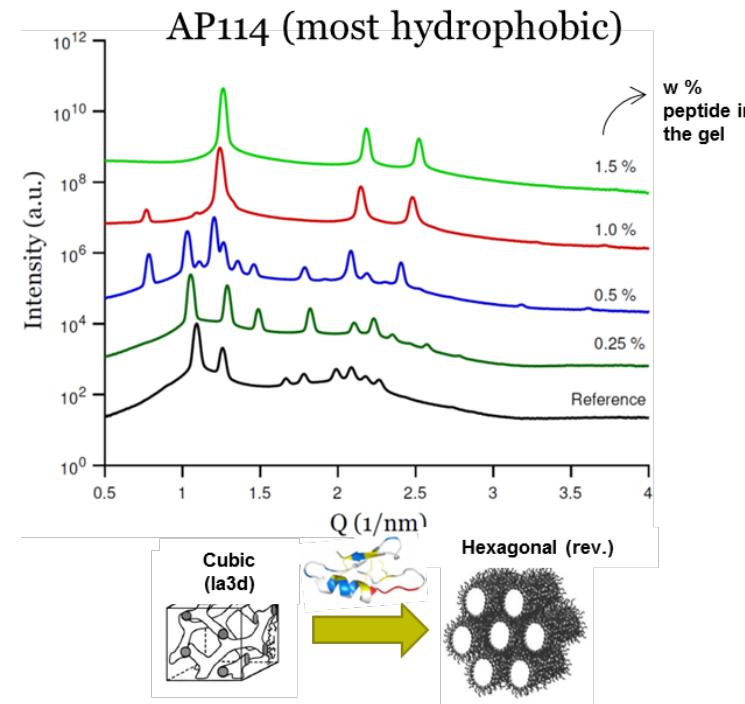


Liquid crystalline nanoparticles (LCNPs) for peptide delivery

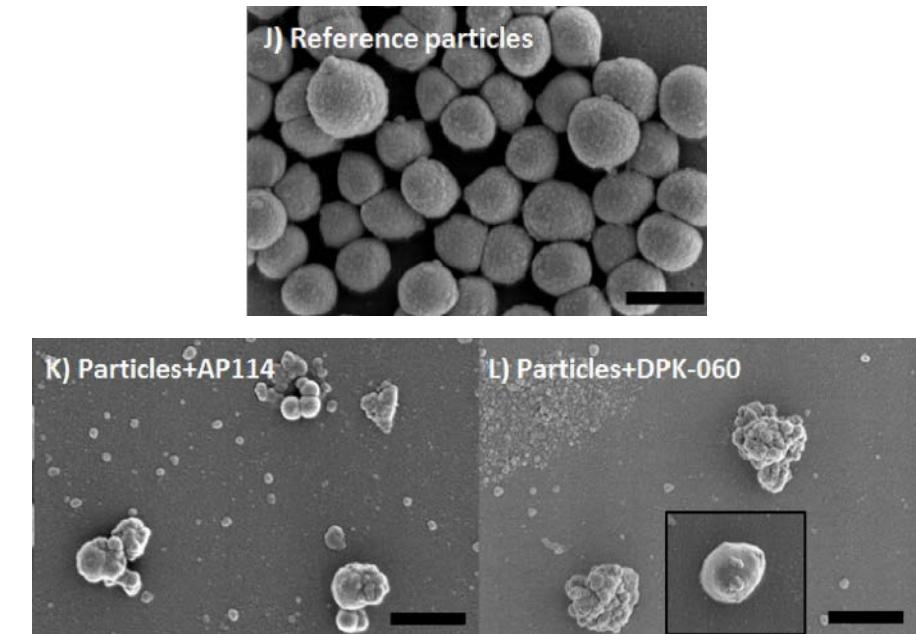
Encapsulation



Phase properties



Antibacterial effect



AMPs can be loaded into the LCNPs with high loading efficiency
The peptide influence the structure of the particles
The antimicrobial effect is maintained
LCNPs prevents sensitive AMPs from proteolytic degradation

Boge et al. 2016, Langmuir

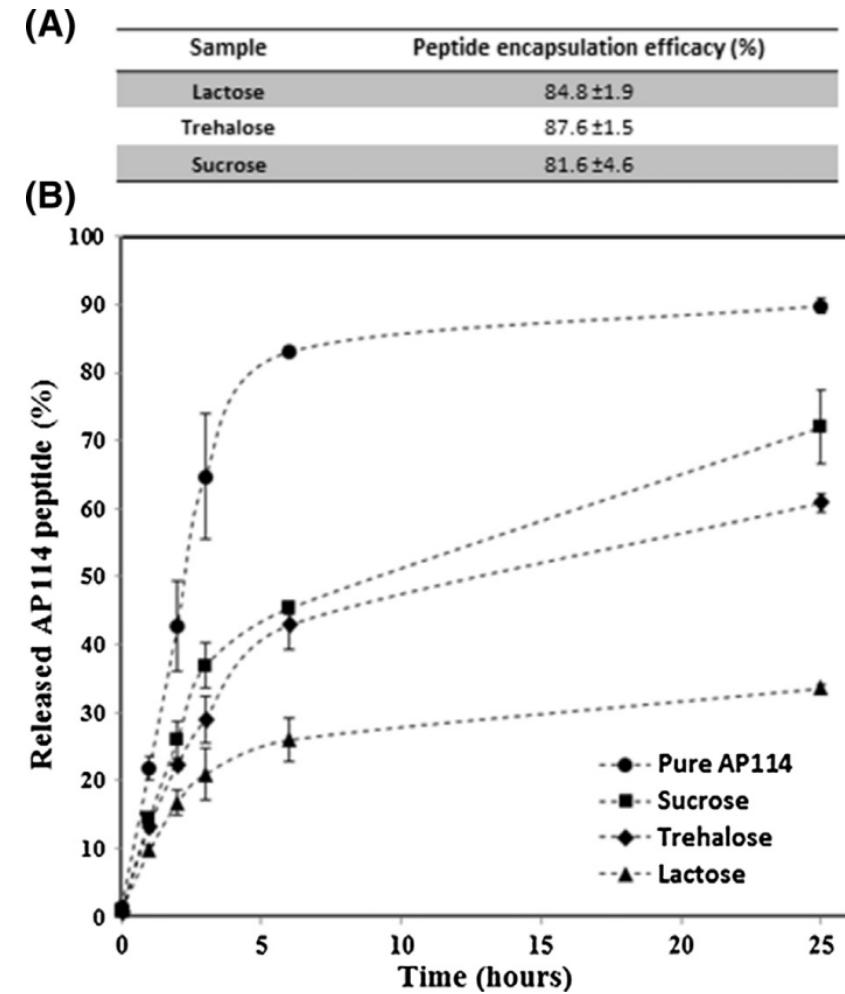
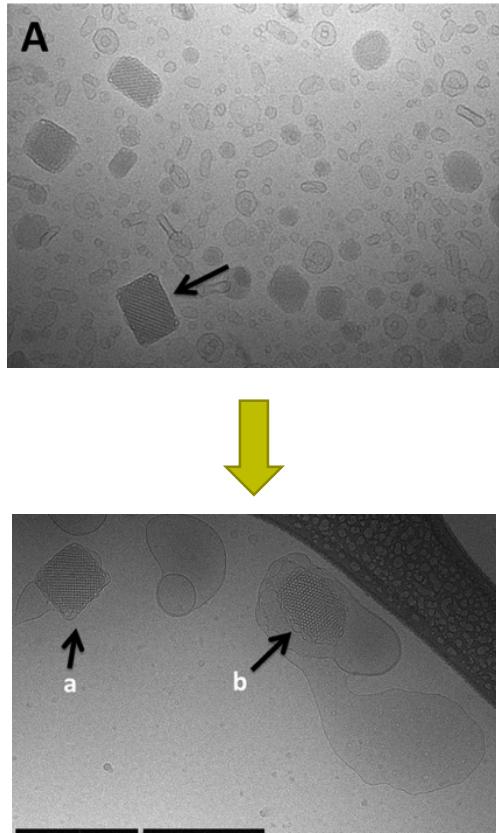
Boge et al. 2017, Int. J. Pharmaceutics

Freeze-dried LCNPs with peptide

Manufacturing process

- Prepare hexosomes/cubosomes
- Mix with excipients
- Freeze-dry
- Redisperse

LCNPs can be freeze-dried and largely maintain structure
The antimicrobial effect is maintained



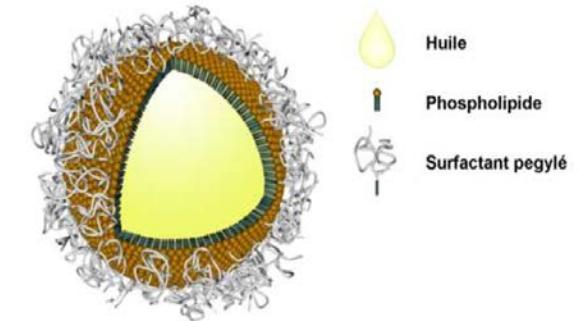
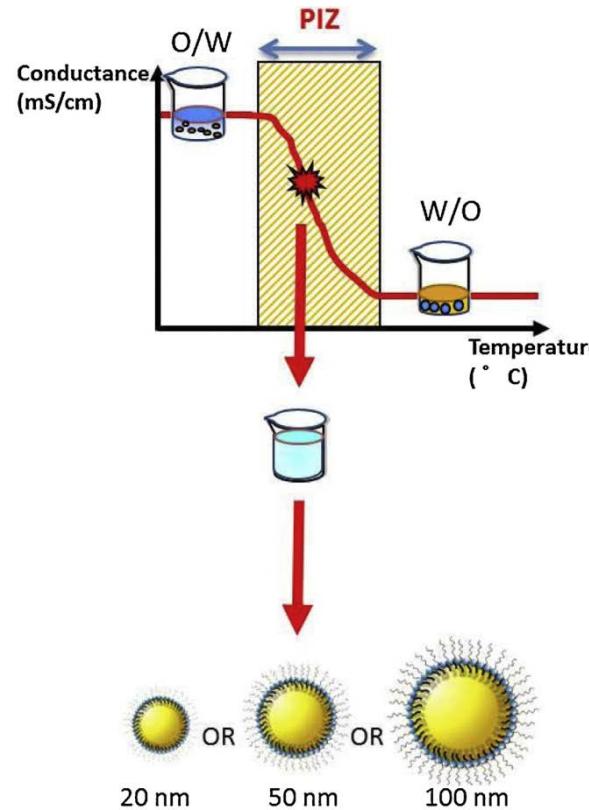
Lipidic nanocapsules

1- Mix of the components

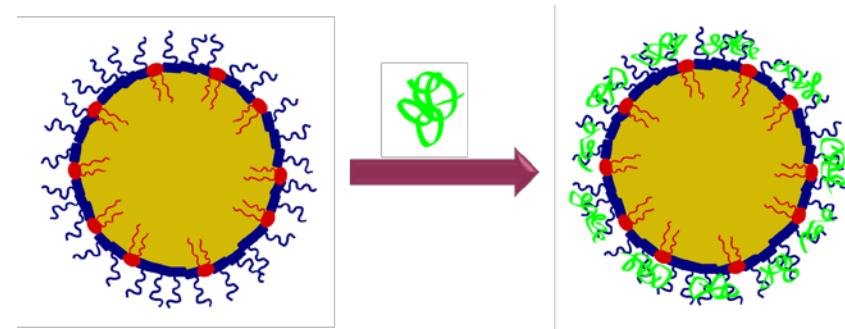
2- Temperature cycles (60 to 90°C)

3- Rapid cooling in PIZ (water 4°C)

4- Stirring



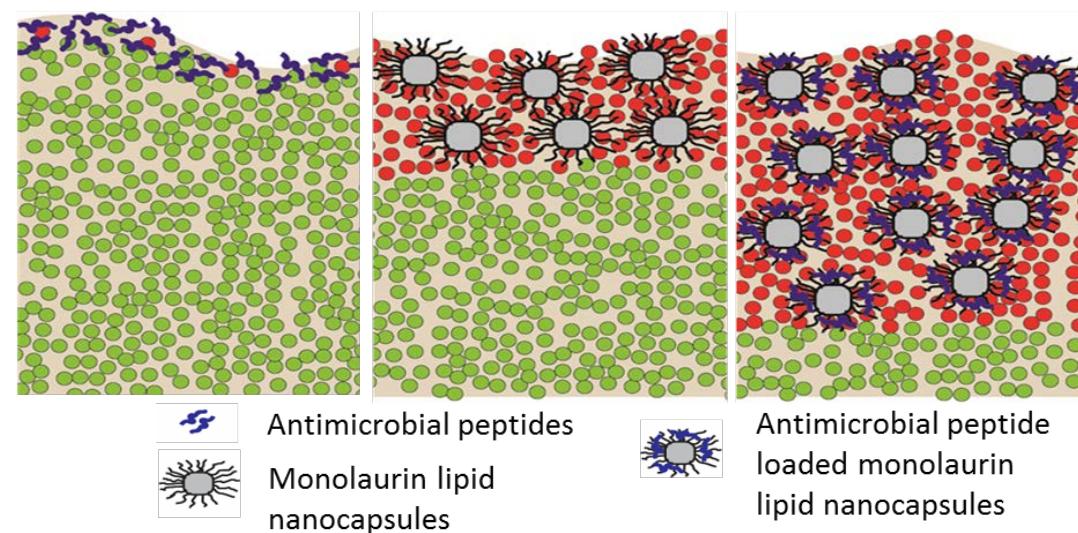
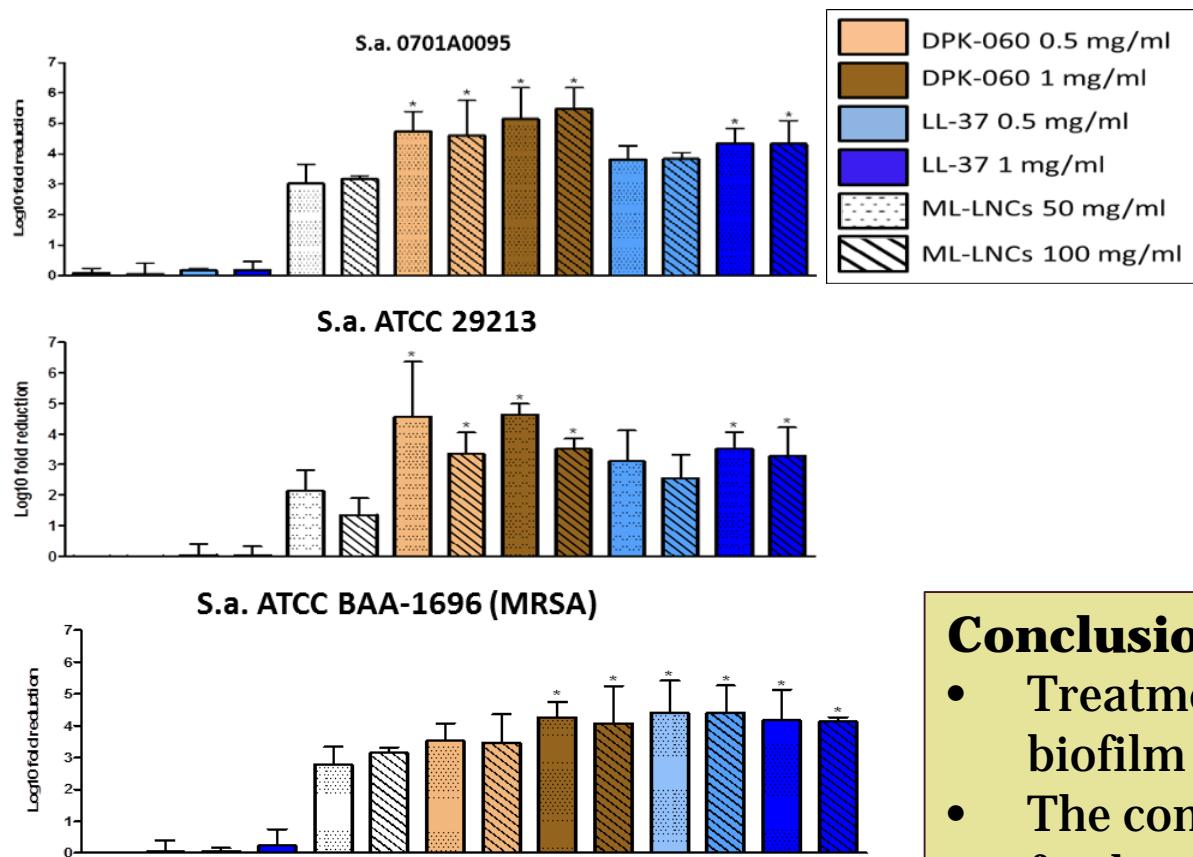
Peptide association driven by electrostatic (and hydrophobic) interactions between LNCs (neg charged) and peptide (pos charged)



- Kolliphor®
- Lipoid®
- Labrafac™
- Peptide



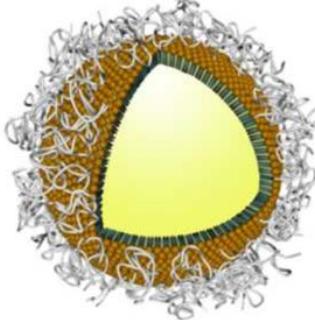
Lipidic Nanocapsules (LNCs) for Biofilm degradation



Conclusions

- Treatment with ML-LNCs alone result in significantly higher biofilm killing compared to AMP alone
- The combination of ML-LNCs and AMPs increase the killing further in most cases – related to diffusion in biofilms
- Combination of AMPs and LNC resulted in clinically relevant reduction.

Up-scaling of LNCs for topical delivery



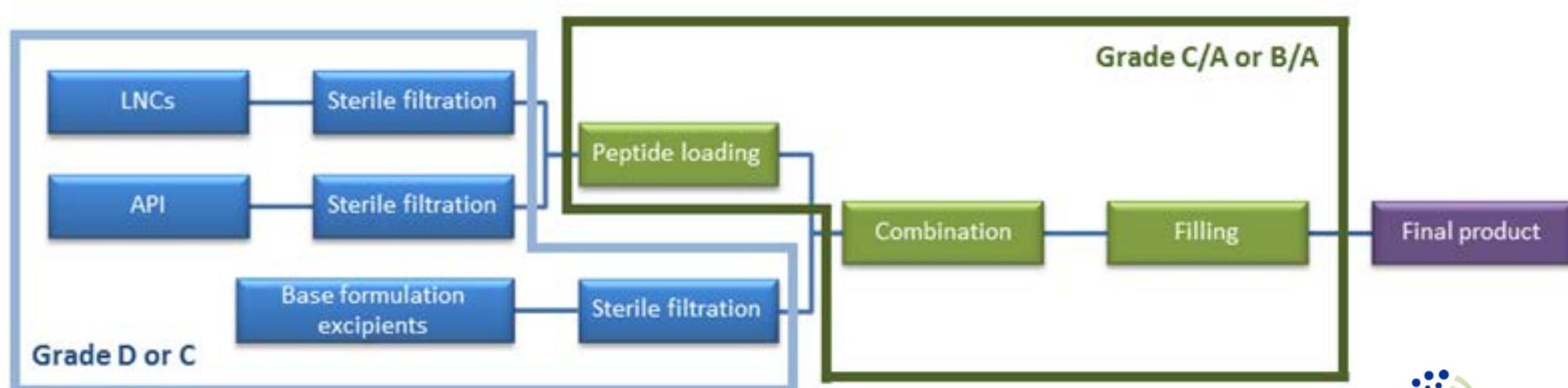
Application to non-intact skin → *sterile formulation required*

Manufacturing process where different levels of clean rooms required for each step (to reduce costs, improve safety) – as large part as possible outside aseptic part

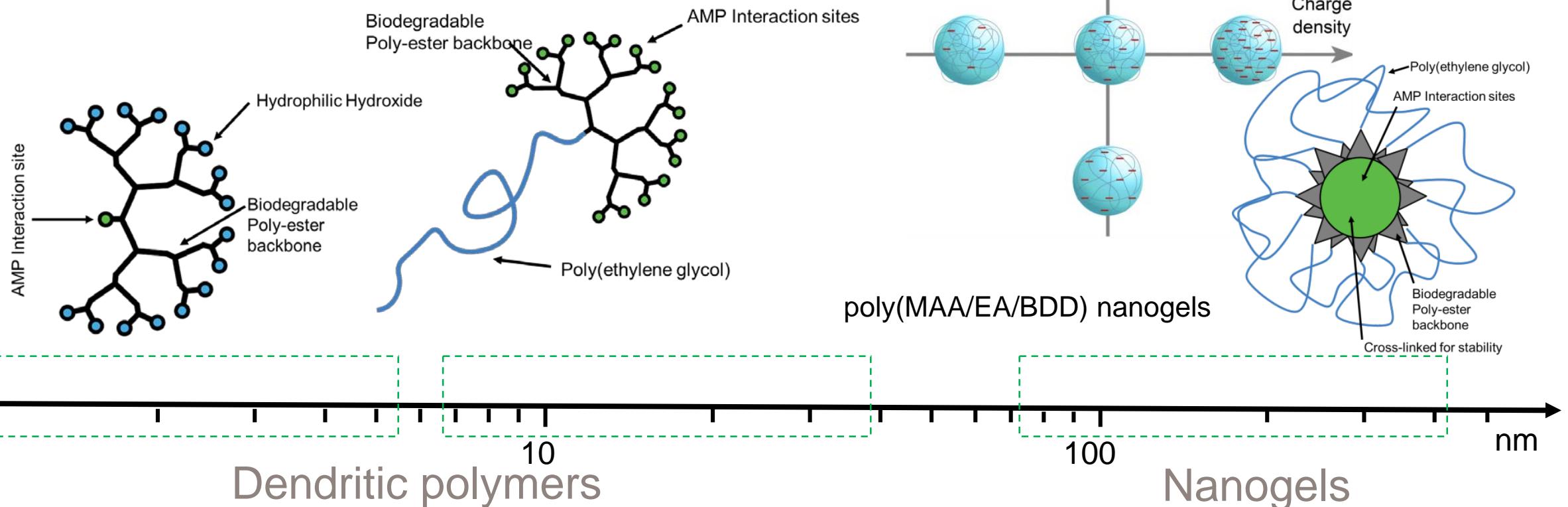
50 times scale-up performed successfully (500 times for LNCs) = up to 10 L scale

Different methods for LNC-manufacturing tested (for further scale-up) – same properties of particles

Successful bioburden studies, LNCs (Total aerobic microbial count, TAMC)



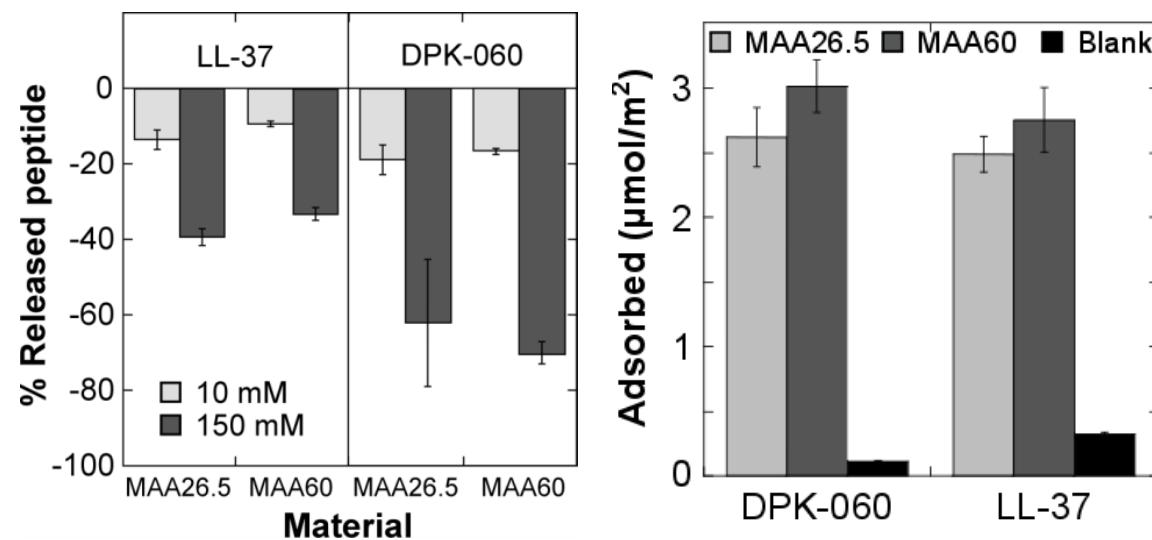
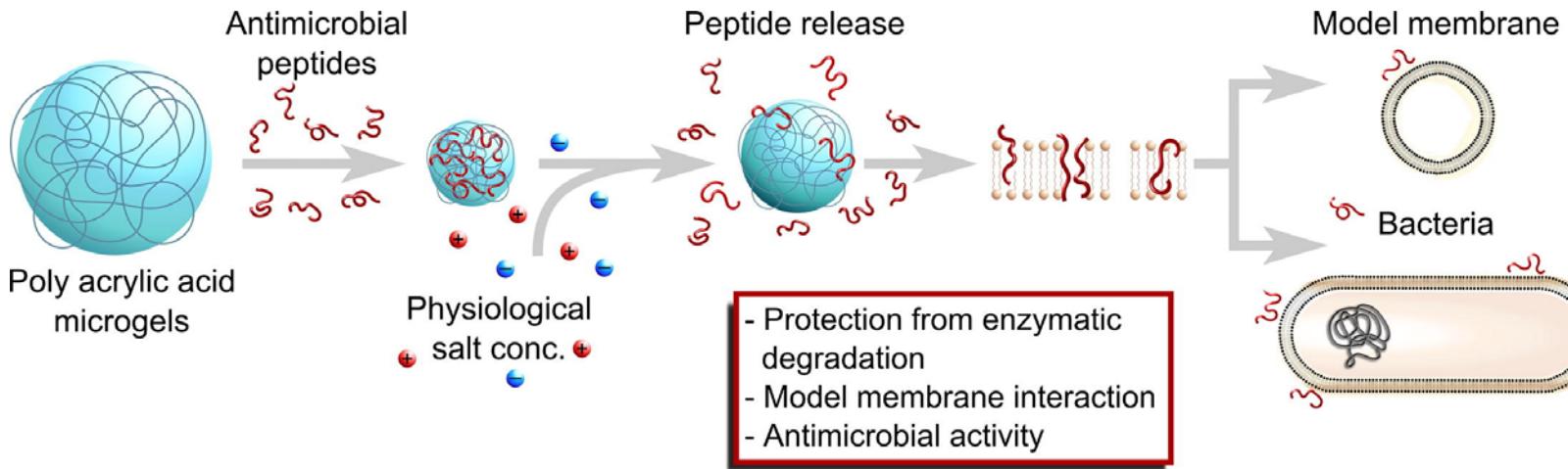
Polymer-based nanoformulations



- Well-defined
- Precise control of size, composition and structure
- Loading capacity can be monitored
- Biocompatible
- Controlled degradation/release feasible

- Hydrophilic, prevent aggregation of peptides
- High loading capacity of AMPs
- Stimuli-responsive release

Polymer-based nanoformulations - nanogels



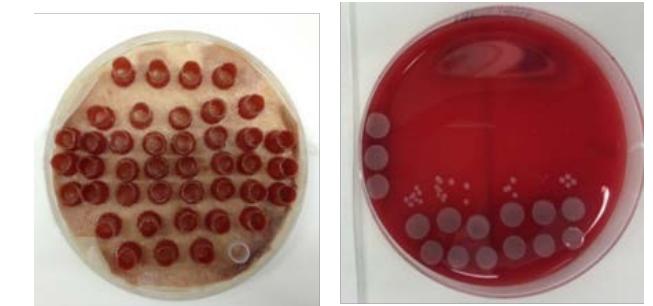
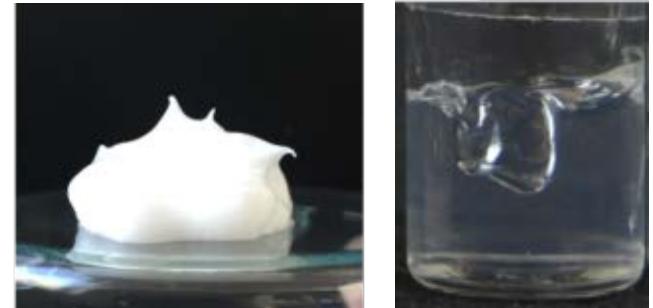
Conclusions

- MAA-gels show high loading capacity and salt induced peptide release.
- MAA-gels reduced the peptide toxicity (hemolysis).
- Nanogel formulation protect the LL-37 from proteolytic degradation.
- Nanogels improved the antimicrobial effect of DPK-060 against two different *pseudomonas* strains.

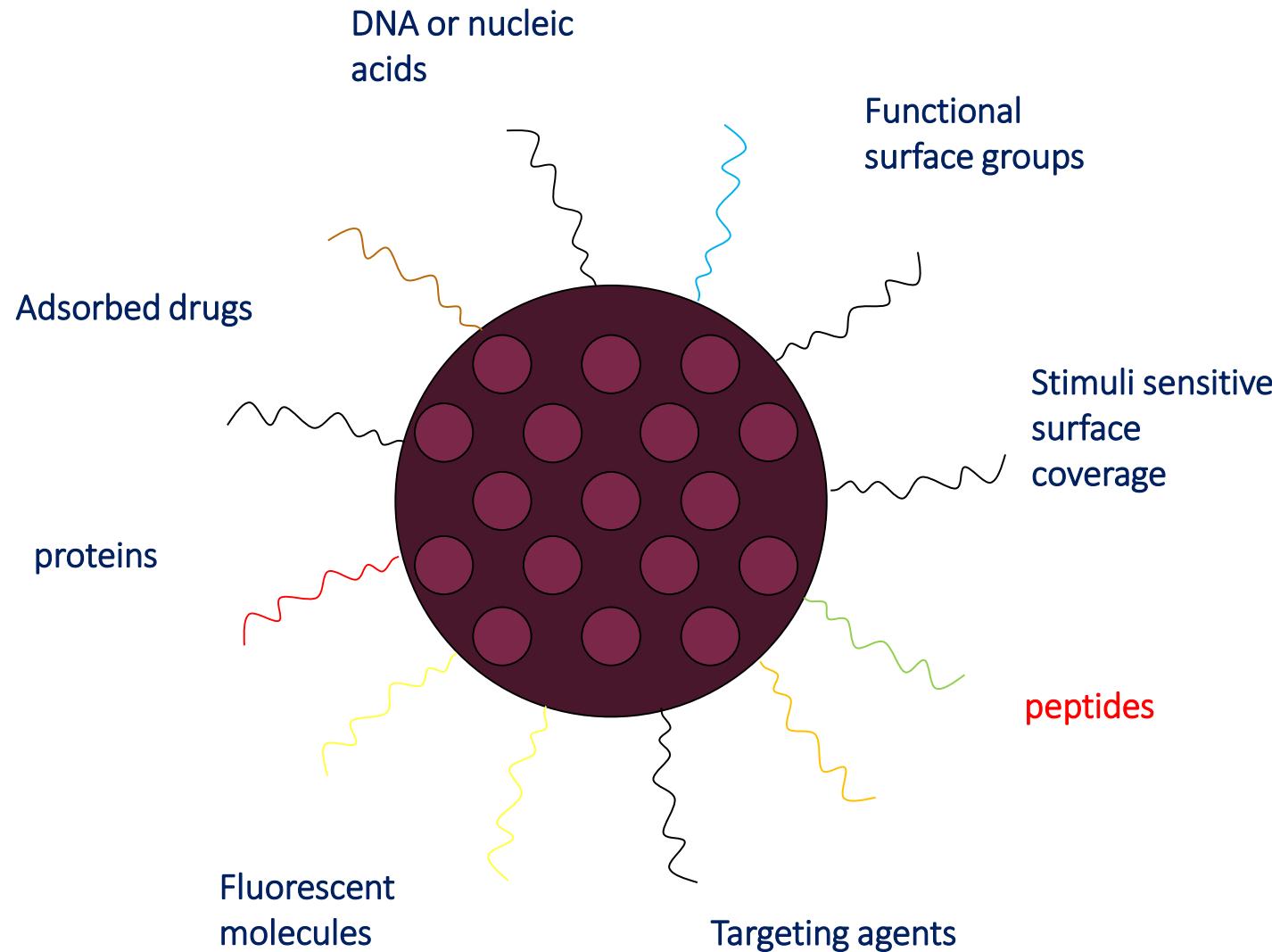
Topical delivery of AMPs

Key project results

- The nanocarriers with encapsulated peptide can be formulated in prototype product formulations (gels and creams) for administration to the skin
- The lipid-based nanocarriers (LCNPs and LNCs) and dendrigels are most suitable in topical formulations
- Dose-response antibacterial effect has been demonstrated in an *ex vivo* wound infection model, where formulations with LNCs and dendrigels presented the most pronounced effect
- The formulations do not cause skin irritation
- LL-37 can be protected from proteolytic degradation when associated to LCNPs or LNCs
- An *in situ* gelling topical formulation with LNCs has progressed in the pre-clinical development pipeline with respect to process development and the scale-up of the manufacturing

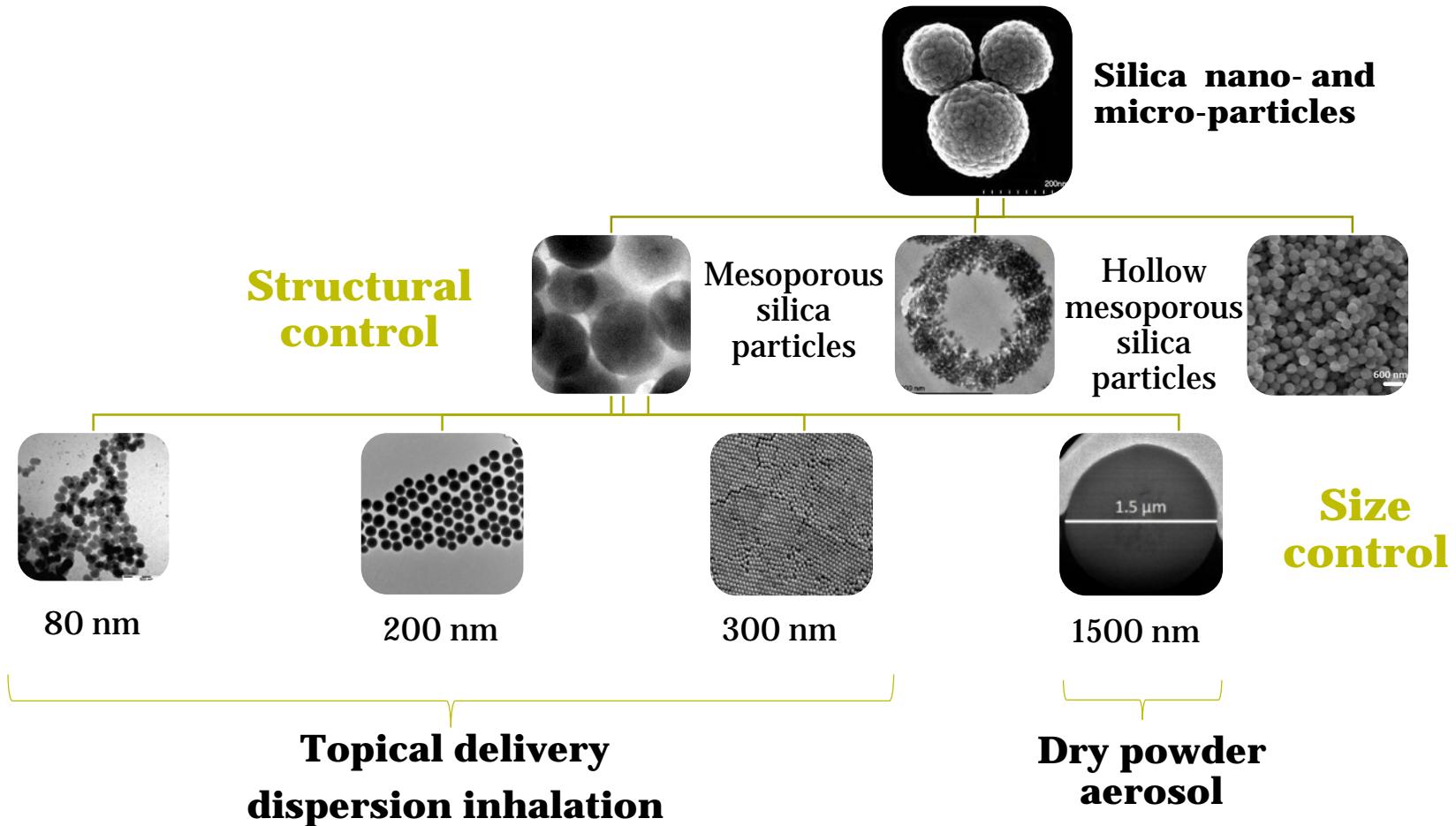


Silica nanoparticles as carrier material for biomedical applications



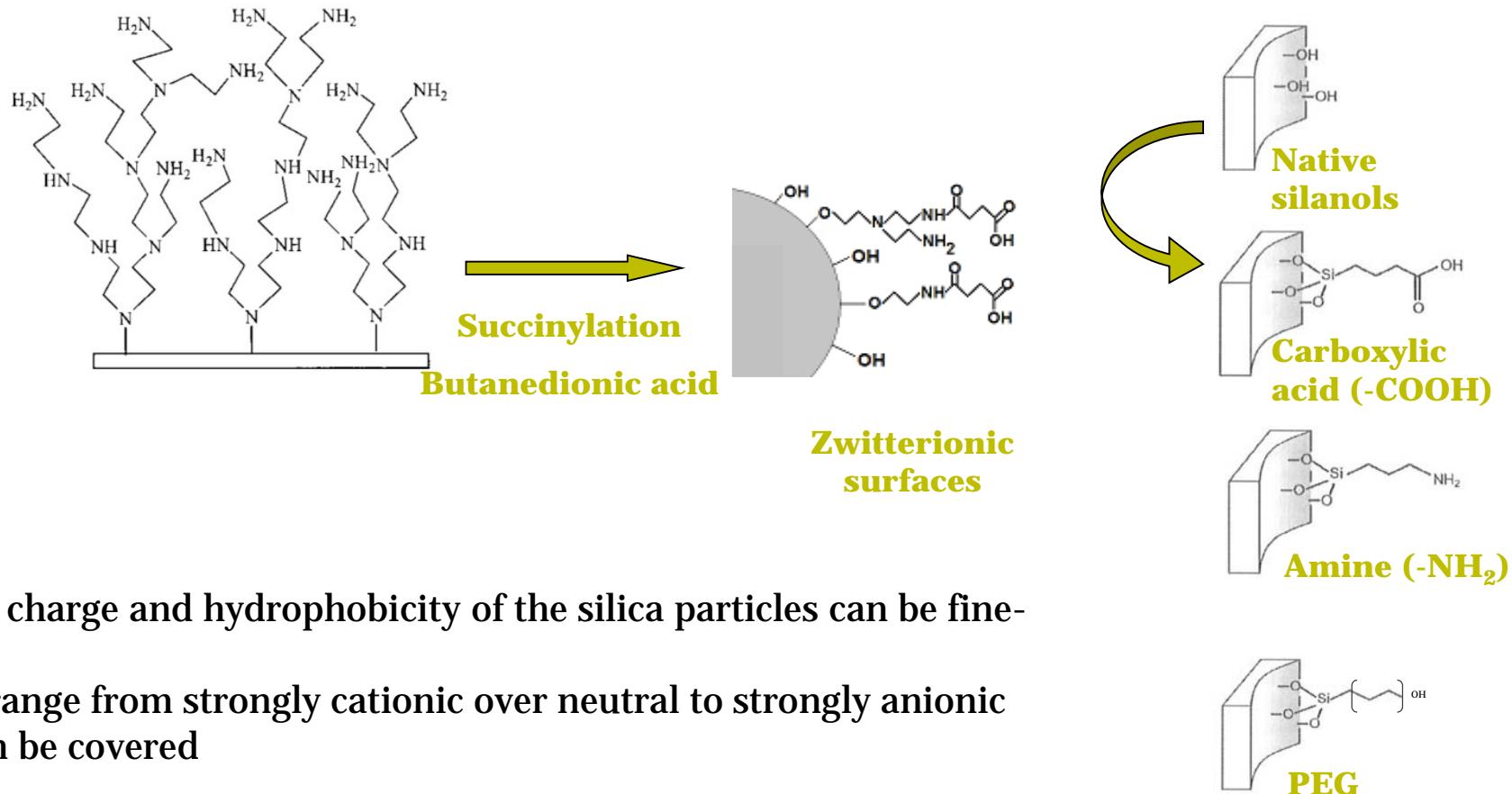
- High loading capacity
- Targeting to different organs
- Particle size and shape control
- Easy surface functionalization
- Release mechanisms

Mesoporous silica particles



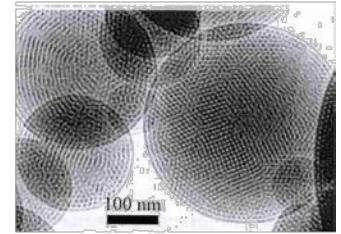
A total of 23 different mesoporous silica particles have been synthesized and characterized in depth as part of the FORMAMP project

Surface chemistry tuning of silica particles



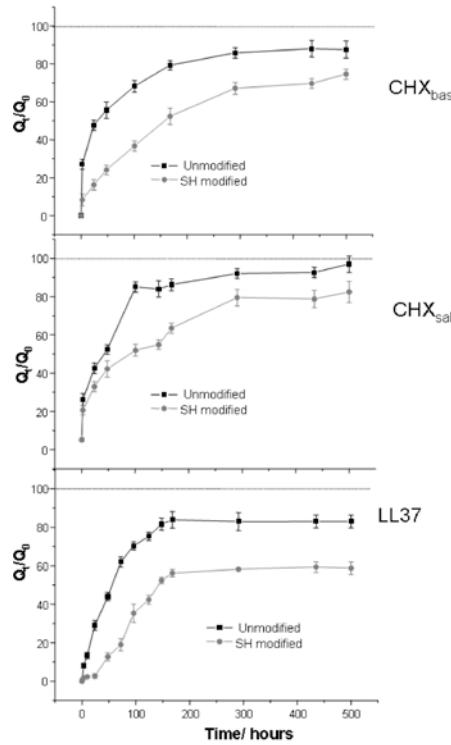
- The surface charge and hydrophobicity of the silica particles can be fine-tuned
- The whole range from strongly cationic over neutral to strongly anionic surfaces can be covered

Nanoformulation of AMPs in mesoporous silica

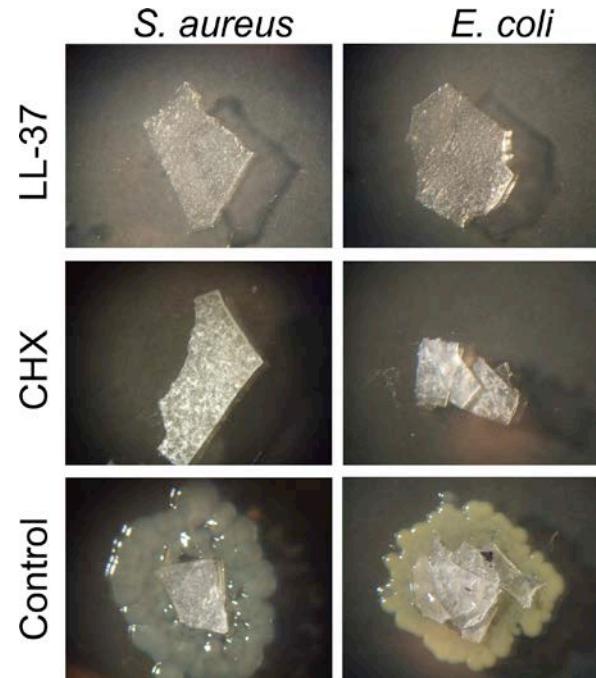


Peptide LL-37 loaded in mesoporous silica to prevent implant-associated infections

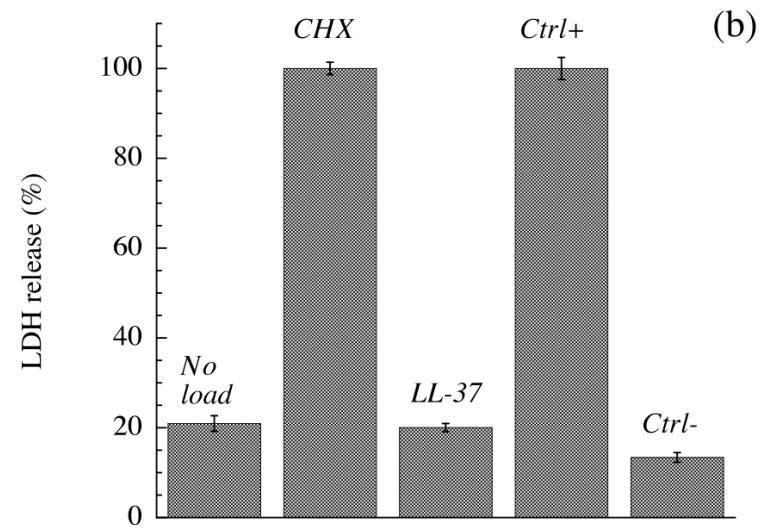
Sustained release



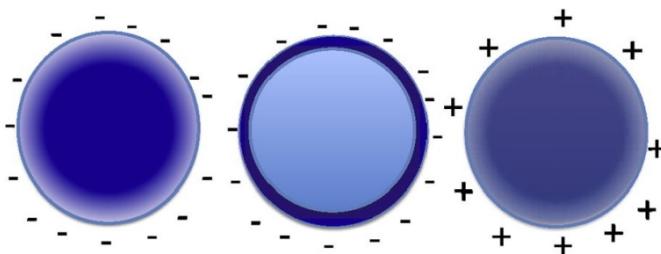
Antimicrobial effect



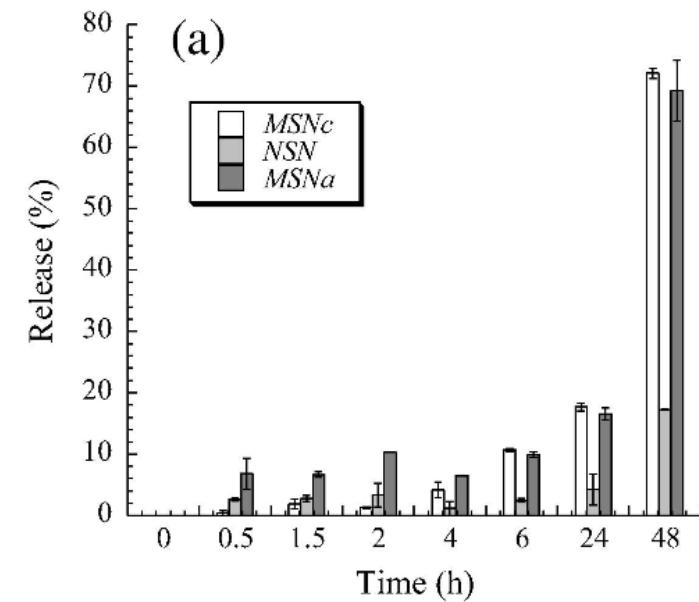
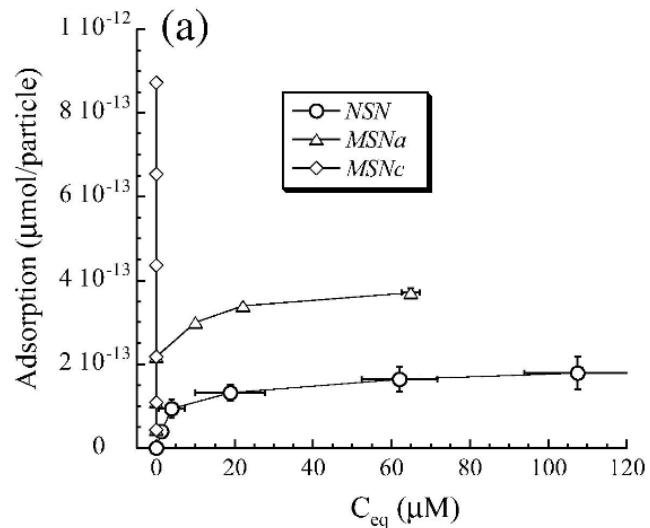
Low toxicity



Nanoformulation of AMPs in mesoporous silica

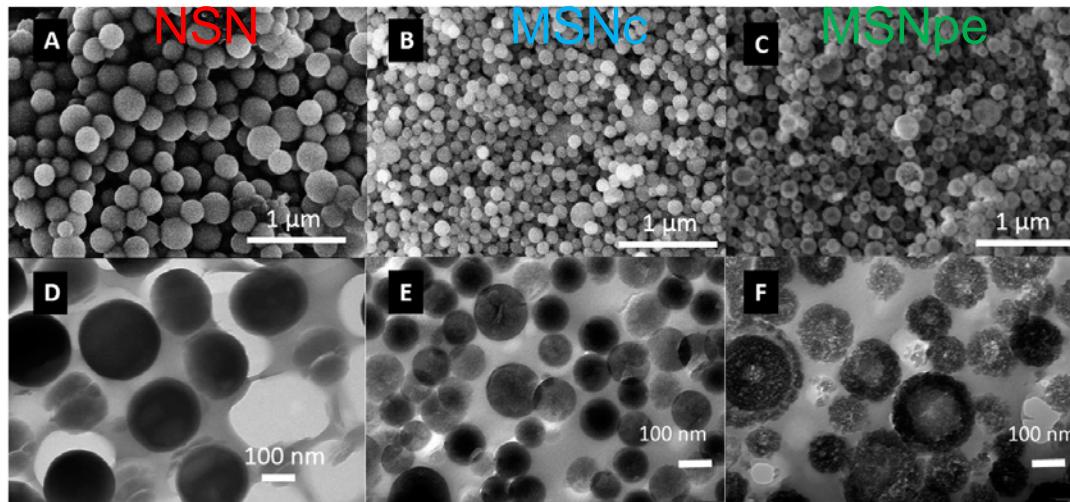
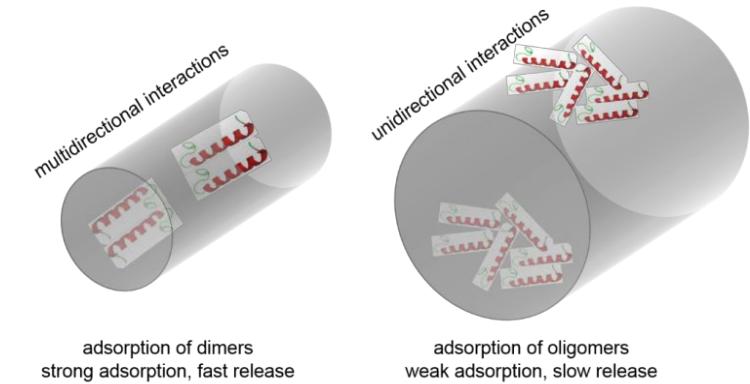
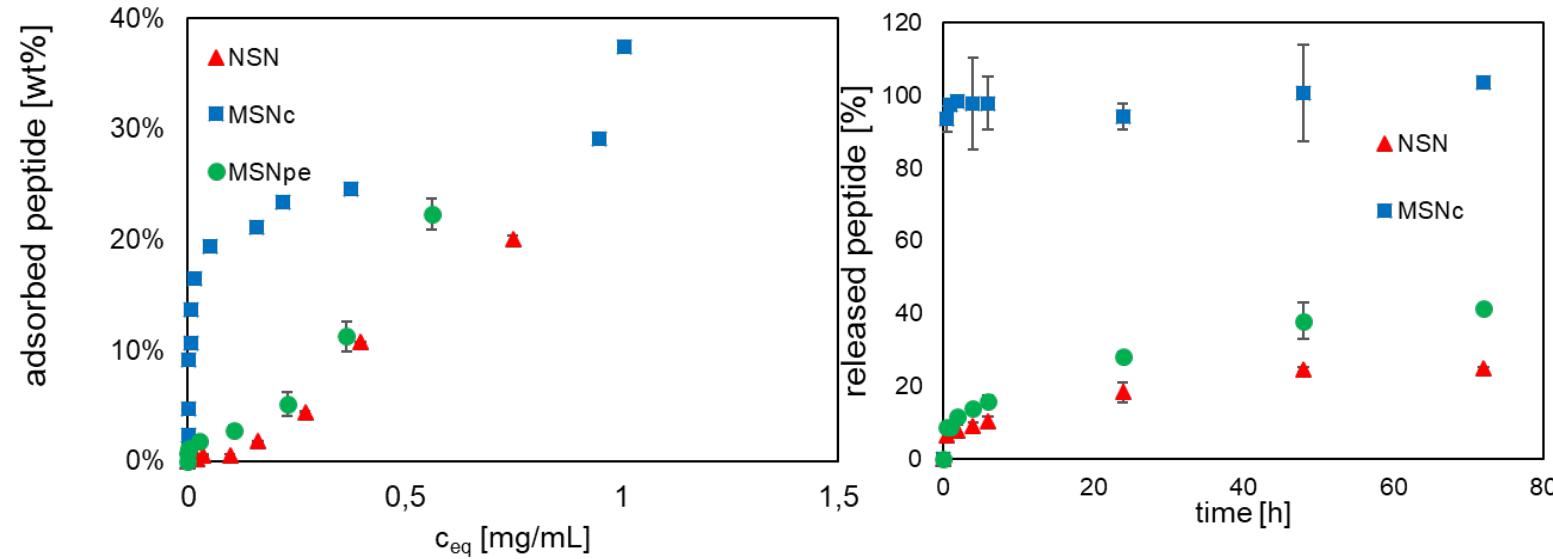


	MSNc (-)	NSN (-)	MSNa (+)
Peptide load	+++++	+	+
Location	Pore	Surface	Pore
Antimicrobial entity	Peptide	Particle	Particle
Proteolytic stability	+	-	-
Toxicity	-	-	+



- Surface charge and surface area strongly influences loading and release of LL-37 and thereby membrane interactions and antimicrobial effect
- The release can be triggered by changes in salt concentrations (physiological conditions)
- Peptide localization correlates to proteolytic stability

Influence of pore size and peptide aggregation on adsorption behavior

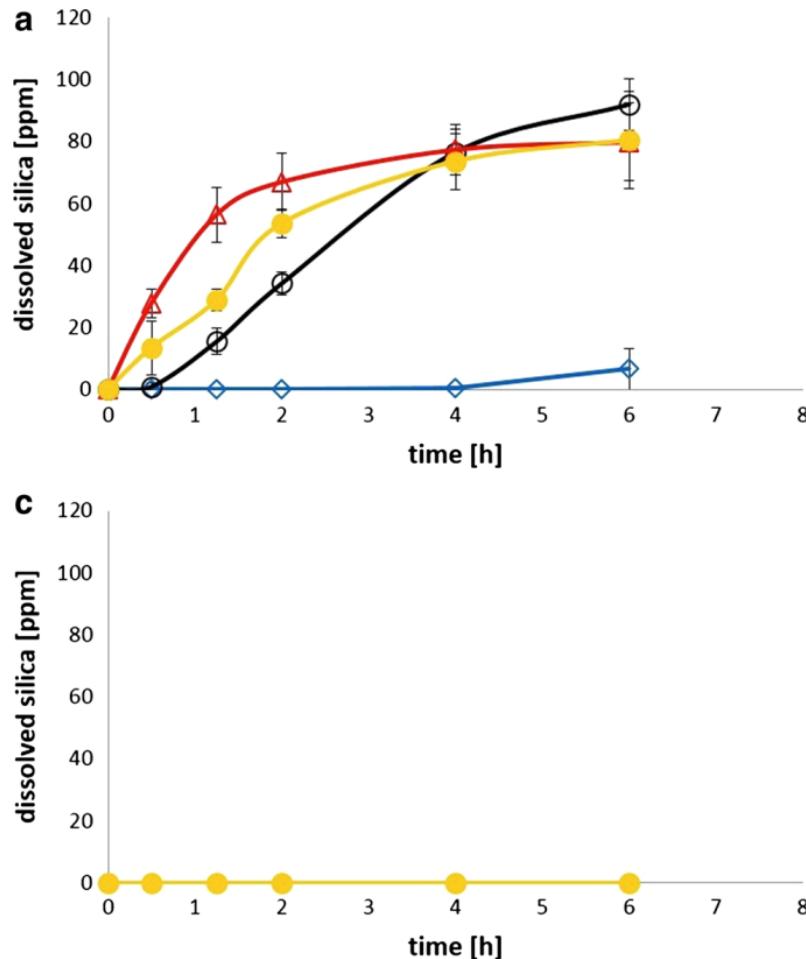


Conclusions

- The pore size and strongly influence the peptide adsorption and release
- The release profile can be fine-tuned by nanoparticle design
- Close match between pore size and peptide size – very strong adsorption and fast release

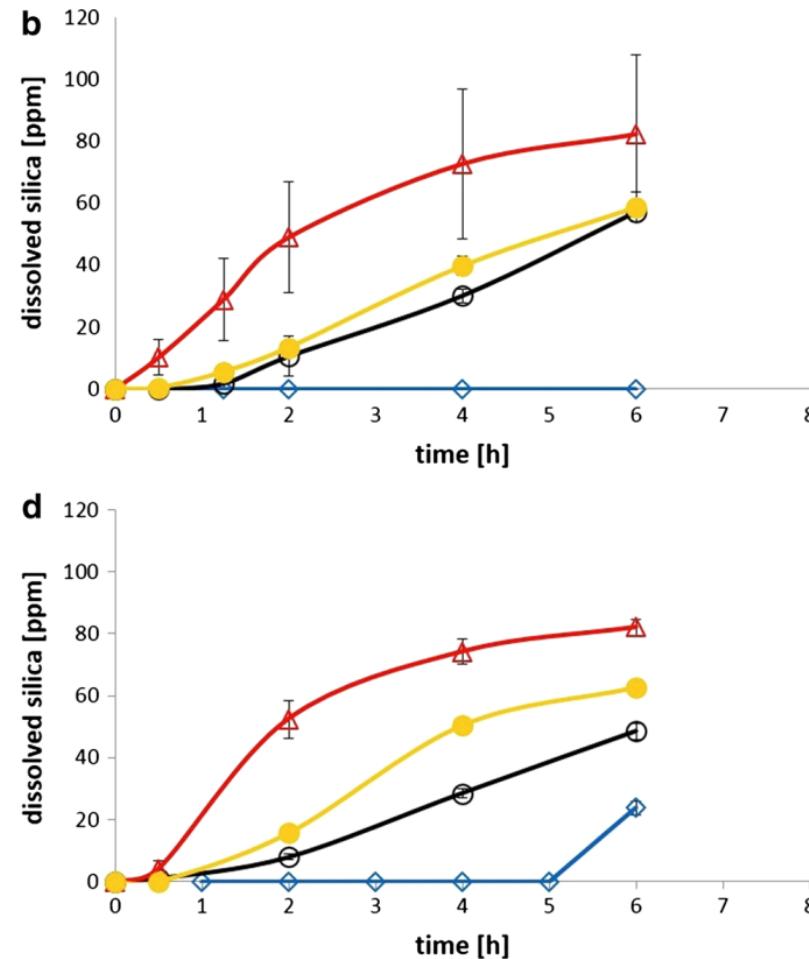
Silica particle dissolution in different media

Simulated lung fluid



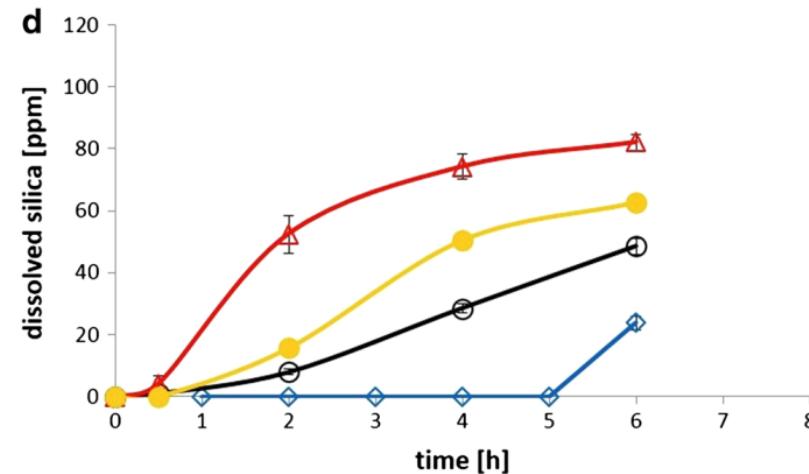
Simulated gastric fluid

- non-porous 200 nm
- mesoporous 80 nm
- mesoporous 200 nm
- mesoporous 1500 nm



Simulated body fluid

PBS



- Silica particle dissolution: SLF > SBF \approx PBS >> SGF
- pH + organic acids strongly influence dissolution

Development of pulmonary tuberculosis treatment

Key project results

- A peptide (NZ61113) capable of killing internalised *M. tuberculosis* in macrophages has been identified
- Peptide can be loaded into different nanocarriers to high loading level
- Nanocarriers can be formulated with additional excipients to form an inhalable powder with required properties for this route of delivery
- Peptide is released into the simulated lung medium
- Nanocarrier have no negative effect on the lung function (*ex vivo, in vivo*)
- The nanoformulated peptide maintains effect on *M. tuberculosis* (*in vitro, in vivo ongoing*)
- Scalable processes for nanocarrier production and loading
- Peptide is stable in the dry nanoformulations





THANK YOU!

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Research Institutes of Sweden

Bioscience and Materials
Surface, Process and Formulation

